Optimisation of the Maillard reaction of bovine gelatine-xylose model using response surface methodology

1,4Ismarti, I., 2,3Triyana, K., 1Fadzilah, N.A., 1Salleh, H.M. and 1,*Nordin, N.F.H.

1International Institute for Halal Research and Training, International Islamic University Malaysia, Jln. Gombak 53100 Selangor Darul Ehsan, Malaysia
2Physics Department, Universitas Gadjah Mada, Yogyakarta, Sekip Utara, BLS 21, Yogyakarta, Indonesia
3Institute of Halal Industry and System (IHIS) Universitas Gadjah Mada, Jl. Kaliurang Km. 4, Sekip Utara, Yogyakarta, Indonesia
4Department of Mathematics Education, Faculty Teaching Training and Education, Universitas Riau Kepulauan, Jl. Batuaji Baru No.99, Batam, Indonesia

Article history:
Received: 19 August 2019
Received in revised form: 23 October 2019
Accepted: 3 November 2019
Available Online: 10 February 2020

Abstract
The Maillard reaction is known as an amino-carbonyl reaction or non-enzymatic browning reaction which has an essential role in food processing to improve the appearance, taste and functional properties of food. In halal authentication, results could be used to differentiate the sources of gelatine based on the colour and flavour. Since many factors can influence the reaction, it is important to study and optimize the Maillard reaction in a gelatine model system using response surface method, applied to optimize the processing of bovine gelatine-xylose to improve the Maillard reaction products. In this study, the effects of initial pH, temperature, and heating time to browning intensity of melanoidin were evaluated. The increasing of initial pH, temperature and heating time were associated with an enhanced browning intensity of Maillard reaction products. This study demonstrated that the coefficient of determination 0.8429 reveals the response surface reduced linear model is an adequate model for browning intensity of Maillard reaction of the bovine gelatine-xylose system. For a system with 5% of gelatine solution and 0.75 g of xylose, the optimum condition for the browning process obtained was initial of pH 10.92, temperature of 140°C and heating time of 37.28 mins. The predicted results at optimum conditions coincided well with the experimental value with the relative error of less than 5%.

1. Introduction
The Maillard reaction is known as a non-enzymatic browning reaction. It refers to a chemical reaction that occurs between the carbonyl groups of reducing sugars and the amino group of amino acids, peptides or protein (Wang et al., 2011). This reaction has played an important role in improving the appearance and taste of food since the Maillard reaction is related to aroma, taste and colour. Also, a wide range of reaction products is formed during the Maillard reaction which can occur in stages. The first stage is the initial stage, which starts with sugar-amine condensation followed with Amadori rearrangement if the sugar is aldose and Heyns rearrangement if the sugar is ketose. The products in the initial stage are colourless without absorption in the ultraviolet spectrum (about 280 nm). The second stage is the intermediate stage, which includes sugar degradation, sugar fragmentation and amino acid degradation (Strecker degradation). In this stage, the colourless or yellow product with strong absorption in the ultraviolet spectrum is formed. In the final stage, aldol condensation, aldehyde-amine condensation, and formation of heterocyclic nitrogen compounds are involved. Products in this stage are highly coloured with a compound called melanoidin (Nursten, 2005).

The colour formation is the primary characteristic of the Maillard reaction which can be readily at the absorbance in the visible region of between 360 and 420
nm. The typical shape of the absorption curve of soluble crude Maillard reaction products is featureless and asymptotic where the absorption is strong at below 400 nm (Nursten, 2005; Rizzi, 2011), but gradually decrease at the higher wavelengths (Nursten, 2005). According to Rizzi (2011), the absence of discrete absorption maxima in the visible region has been interpreted as the result of a polymerization process.

Melanoidins are polymers with a high molecular weight of about 1500 kDa which usually contains 3-4% nitrogen (Nursten, 2005), and some residual protein (Rizzi, 2011). Food melanoidins are anionic compounds predominantly responsible for the characteristic brown colour of food such as coffee, cocoa, bread, malt and honey (Wang et al., 2011). The complex array of melanoidins produced in the Maillard reaction is strongly dependent on the types of food, as well as the technological conditions of the reactions such as treating temperature and time, pH, solvent, and the compositions of the amino acids and reducing sugar (Van Boekel, 2006; Jaeger et al., 2010; Wang et al., 2011). According to Kwak and Lim, (2004), the colour intensities of MRPs containing basic amino acids was reported greater than that of acidic amino acids, while nonpolar amino acidic have intermediate colour intensities. In addition, browning was accelerated by the presence of metal ions (Fe$^{2+}$ and Cu$^{2+}$) but inhibit by Na$^+$. Gelatine has been used in a wide range of food products. MRPs of gelatine is one of the special interests in halal authentication since these products allow differentiation of the gelatine based on its origin and sources. The sources of gelatine in the market are mammal gelatine, mainly from porcine and bovine — also, little supply from fish. However, the unclear labels of gelatine in the market in terms of their sources raise doubts among Muslim consumers, since the Muslim are prohibited from consumption of porcine derivatives. Study on the Maillard reaction for differentiation of gelatine has been reported by Tan et al., (2012) where the bovine and porcine gelatines were successfully differentiated with UV-spectroscopy. In 2017, Hamizah et al. reported that the presence of Cu$^{2+}$ in the Maillard reaction of gelatine causes an increased rate of browning. Also, our previous study has successfully established the method for classification of bovine, porcine and fish gelatin bases on flavours using an electronic nose. In the study, the Maillard reaction has enhanced the accuracy of the method since it improved the flavour of gelatine.

In this study, optimization of Maillard reaction from the gelatine-xylose model was conducted since it influences sensory characteristics such as colour, aroma and taste possible as use for gelatine authentication.

## 2. Materials and methods

### 2.1 Chemicals

Chemicals used were bovine skin gelatine type B and xylose, purchased from Sigma Aldrich, Germany, sodium hydroxide and hydrochloric acid from Merck, and ultrapure water. All chemicals used were of analytical grade.

### 2.2 Screening of variables influence the browning intensity

The first step in the design of the experiment is screening and choosing the process parameters, and the response variables for optimization using one factor at-a-time (OFAT) method. According to previous experimental findings, the most influential factors of Maillard reaction are gelatine concentration, xylose concentration, initial pH, reaction temperature, and heating time. The browning intensity indicates the MRPs formation and was measured at an absorbance of 360 nm.

#### 2.2.1 Gelatine solution concentration

A series of gelatine solution (5, 10 and 15% (w/v)) were prepared by dissolving standard bovine gelatine in 100 mL ultrapure water. The mixtures were homogenized at 60°C using a hotplate stirrer. The solution was cooled at room temperature for further step. The pH of the solution was determined with a pH meter.

#### 2.2.2 Xylose concentration

About 5 mL of gelatine solution was put into a screw-sealed tube. A variation weight of xylose (0.25 g, 0.50 g, 0.75 g, and 1.00 g) was added into the solution. The tube was tightly capped, homogenized and heated in a water bath at 90°C for 60 mins. After heating, the samples were immediately placed in an ice bath to stop the further reaction. The browning intensity was determined following the method of Liu et al. (2016). Appropriate dilution (10-fold) of the MRPs was made, and the absorbance was measured at 360 nm using a USB4000 UV-Visible Spectrophotometer with Ocean View software 1.5.0 version. All samples were prepared in duplicates.

#### 2.2.3 Initial pH

In order to evaluate the effects of the initial pH, a series of 5% gelatine solution were prepared at different pH of 4, 6, 8, 10 and 12 using 1M HCl or 1M NaOH. About 5 mL of the gelatine solution was mixed with 0.75 g of xylose in a screw-sealed tube, homogenized and...
heated at 90°C for 60 mins. The browning intensity was measured as in Section 2.2.2.

2.2.4 Reaction temperature

About 5 mL of gelatine solution (5% w/v) from the optimum initial pH was prepared with 0.75g of xylose. The mixture was heated at different temperatures (70, 80, 90, 100, 110, 120, 130, 140 and 150°C) for 60 mins to evaluate the effect of temperature on browning intensity. The browning intensity was measured as in Section 2.2.2.

2.2.5 Heating time

About 5 mL of gelatine solution (5% w/v) from the optimum temperature was prepared with 0.75 g of xylose. The mixture was heated at 140°C with the variation of heating time (0, 15, 30, 45, 60 and 75 minutes) to evaluate the effect of heating time on browning intensity. The browning intensity was measured as in Section 2.2.2.

2.2.6 Statistical analysis

Effect of variables on the browning intensity as the representation of Maillard product was analyzed using one-factor-at-a time (OFAT) method. All of the measurements on each sample were carried out in duplicate. The results were shown in mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA) using Microsoft Excel 2010. Mean values were compared using Tukey’s test at P<0.05.

2.3 Optimization of Maillard reaction model

2.3.1 Design of experiment

In order to evaluate interactions between initial pH, temperature and heating time, Response Surface Methodology (RSM) with Central Composite Design (CCD) was used in designing the experiment. The design was constructed using Design Expert Version 6.0.8 leading to 17 sets of experiments, allowing each experimental response to be optimized. The experimental design of the coded (X) and actual (initial pH, reaction temperature, and heating time) level of variables are shown in Table 1. Each independent variable had coded levels of -1 (low level) and +1 (high level). The responses functions Y was related to coded variables \(X_i\) (\(i = 1, 2, 3, 4\)) by the following second-order polynomial equation (Zhang et al., 2016):

\[
Y = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \sum_{k=j+1}^{4} b_{ijk} X_i X_j X_k
\]

(1)

Where Y represent browning intensity; \(b_0\) represent constant; \(b_i\) represents the regression coefficient for linear effect; \(b_{ij}\) represents the quadratic coefficient, and \(b_{ijk}\) represents the interaction coefficient.

The Maillard reaction model system was prepared based on results obtained from Section 2.2. The system consisted of about 5 mL of gelatine solution (5% w/v) and 0.75 g of xylose. The mixture was put into screw-sealed tubes, tightly capped and heated in a water bath according to the experimental design in Table 1 in duplicates. After heating, the samples were immediately placed in an ice bath to stop further reaction. The obtained MRPs samples were determined using UV Visible spectrophotometer at 360 nm. The average values were recorded as the response.

| Level code | Variable | Variable level |
|------------|----------|----------------|
| X1         | Initial pH| 10 12          |
| X2         | Temperature (℃)| 120 140 |
| X3         | Heating time (min)| 30 45 |

2.3.2 Statistical analysis

Analysis of variance (ANOVA) was performed using Design Expert Version 6.0.8 where ANOVA tables were generated, and the effect and regression coefficient of individual linear, quadratic and interaction terms were determined. The statistical significance of the regression coefficient was determined by using F-test and lack of fit test, while the applicability of the model was checked with significance coefficients of determination (R²) values. The optimum processing conditions were obtained by using numerical analysis based on the criterion of desirability.

2.4 Validation model

Validation model was performed based on the conditions recommended by the Design-Expert software. The measurement was performed in triplicate. The mean ± SD of results from the experiment were compared with the predictive value from the model to get the percentage of relative error by using the Equation (2).

\[
\text{Relative error} = \left| \frac{\text{predictive value} - \text{actual value}}{\text{predictive value}} \right| \times 100\%
\]

(2)

3. Results and discussion

Standard bovine gelatine was used in this study. Xylose was used in this model because of its high reactivity and relatively lower cost compared to other hexoses. As many factors can influence the Maillard reaction, CCD using RSM was applied to determine the best conditions. Response surface methodology is a collection of statistical and mathematical techniques useful for the improvement and optimization of complex processes. The main advantage of RSM is its ability to reduce the number of experimental trials needs to
evaluate multiple parameters and their interaction to provide sufficient information for statistically acceptable results (Gu et al., 2009; Burin et al., 2013).

3.1 Effect of variables measurement on browning intensity

3.1.1 Gelatine solution concentration

In this study, gelatine solution with variation in concentrations 5, 10 and 15% (w/v) was used. The pH of the gelatine solutions at different concentrations is shown in Table 2. Statistical analysis using ANOVA for OFAT method indicated that the calculated F value (0.64) was less than tabulated F value (9.55) and P-value higher than 0.05. It means that there is no significant difference between the pH of gelatine solution with a variation of concentration before reaction. However, gelatine solution with 10% (w/v) and 15% (w/v) concentrations are not suitable for use in the experiment since the solution thickness at room temperature. Based on this fact, this study used 5% (w/v) concentration of gelatine for further experiment.

Table 2. pH of gelatine solution at variated concentrations

| Gelatine concentration (%) | pH     |
|----------------------------|--------|
| 5                          | 4.64±0.01<sup>a</sup> |
| 10                         | 4.67±0.04<sup>a</sup> |
| 15                         | 4.66±0.01<sup>a</sup> |

Mean±SD (n=3). Different superscripts within the same column indicate the significant difference (P<0.05).

3.1.2 Xylose concentration

Four variations of weights of xylose (0.25 g, 0.5 g, 0.75 g and 1.00 g) were used to evaluate the effect of xylose on the browning intensity. The results of browning intensity were recorded at the absorbance of 360 nm wavelength. As shown in Table 3, there is increasing in browning intensity with the increasing of xylose weight. Statistical analysis using ANOVA single factors obtained for the calculated F value (73.95) was higher than the tabulated F value (6.59) at alpha 0.05. It means that there is a significant difference for browning intensity with the difference of xylose weight.

Table 3. Browning intensity of Maillard products at variation xylose weight

| Xylose (g) | A<sub>360</sub> |
|------------|----------------|
| 0.25       | 1.08±0.07<sup>a</sup> |
| 0.5        | 1.64±0.14<sup>b</sup> |
| 0.75       | 2.09±0.01<sup>c</sup> |
| 1          | 2.13±0.06<sup>c</sup> |

A<sub>360</sub> = Absorbance at the wavelength of 360 nm. Different superscripts within the same column indicate the significant difference (P<0.05).

Further analysis using the Tukey's test obtained HSD<sub>(0.05,3,4)</sub> = 0.28. This value was then compared with the different means of each group. The results of Tukey's test in Table 3 shows that there is a significant difference in browning intensities for variations of xylose except for 0.75 g and 1.00 g of xylose.

3.1.3 Initial pH

To evaluate the effect of initial pH, 5% of gelatine solution mixed with 0.75 g of xylose was used. The browning intensity was determined at the absorbance of 360 nm after heating at 90°C for 60 mins, as shown in Table 4. From the table, it is clear that the browning in the Maillard products increased moderately with the increase in initial pH within the range of 4 to 12. Statistical analysis using ANOVA indicated that there is a significant difference in browning intensities as affected by initial pH, as shown by the F value calculated (67.24) higher than F value tabulated (5.19) at alpha 0.05. However, the Tukey's test showed that there is no significant difference in browning intensity for Maillard products with initial pH of 6, 8 and 10.

Table 4. Effect of initial pH on browning intensity

| Initial pH | A<sub>360</sub> |
|------------|----------------|
| 4          | 0.09±0.03<sup>a</sup> |
| 6          | 0.57±0.05<sup>b</sup> |
| 8          | 0.57±0.08<sup>b</sup> |
| 10         | 0.56±0.06<sup>b</sup> |
| 12         | 0.98±0.04<sup>c</sup> |

Different superscripts within the same column indicate the significant difference (P<0.05).

High initial pH is therefore beneficial to produce Maillard products from bovine gelatine-xylose models as the Maillard reaction is catalyzed in alkaline condition (Nursten, 2005; Gu et al., 2009). Based on the experiment, at initial pH 12, brown colour formed within the first 15 mins of reaction. However, for initial pH 8-10, the brown colour formed after 30 minutes while for pH 4 it formed after 45 mins of heating time. The presence of amine or alkaline condition will increase the reaction rate since base or amine can act as a catalyst for Maillard reaction. At the final stage, the pH of Maillard reaction drops from 4.95-4.65. The final stage of the Maillard reaction involves aldol condensation. In this step, aldehydes from the intermediate stage can react with each other. The presence of amine in the system will increase the reaction rate since the amines are effective catalysts. At the end of the process, aldehydes react readily at low temperatures with amines to give polymeric high molecular mass, coloured products of unknown structures, called melanoidins (Nursten, 2005). According to Ames and Apriyantono (1994), pH has an important influence on the profile of products formed during the Maillard reaction.
3.1.4 Reaction temperature

For this study, 5 mL of gelatine solution (5% w/v) at pH 12 (from Section 3.1.3) was mixed with 0.75 g of xylose and homogenized. The solutions were heated at different temperatures for 30 mins. Effect of temperatures on the browning intensities is shown in Table 5. From the results in Table 5, the brown Maillard products were formed at 70°C. The browning increased slightly with increasing temperature and reached the maximum at 140°C. It is therefore clear that the temperature has a significant effect on the browning intensity. It was supported by ANOVA results where the F value calculated (2400.34) higher than F value tabulated (3.23) at alpha 0.05 and P-value < 0.05. According to Nursten (2005), the browning increased 2-6 times with increasing 10°C in temperature. Further analysis using Tukey’s test shows it is significantly different between browning intensity in low-temperature group (70-90°C), middle-temperature group (100-120°C) and high-temperature group (130-150°C). However, there is no significant difference in browning intensity within the group for low-temperature and high-temperature groups.

Table 5. Effect of temperature on browning intensity

| Temperature (°C) | ΔA_{360} |
|------------------|---------|
| 70               | 0.02±0.01^a |
| 80               | 0.04±0.05^a |
| 90               | 0.04±0.01^a |
| 100              | 0.44±0.04^b |
| 110              | 1.05±0.05^c |
| 120              | 1.50±0.02^d |
| 130              | 2.40±0.00^e |
| 140              | 2.45±0.01^e |
| 150              | 2.42±0.04^e |

A_{360} = Absorbance at the wavelength of 360 nm. Different superscripts within the same column indicate the significant differences (P<0.05).

3.1.5 Heating time

The last variable studied that also influenced the browning in Maillard products is heating time. The browning intensity of Maillard product of gelatine-xylose system at variation of heating time between 0.96 to 2.90 is as shown in Table 6. It is clear that the browning intensity increased slightly within the first 15 mins of heating time and fluctuated after that. Statistical analysis using ANOVA shows that the F value calculated (83.80) was higher than F-value tabulated (4.39) at alpha 0.05 and P-value < 0.05. It means that there is a significant difference in the browning intensity at different heating time.

Further analysis to explore the source of significant difference was done using Tukey’s test. From the results obtained, 0 min of heating time showed a significant difference from other heating times. In addition, there was no significant difference in browning intensities at 15, 30, 45, 60 and 75 mins of heating time.

Table 6. Effect of heating time on browning intensity

| Heating time (min) | ΔA_{360} |
|-------------------|---------|
| 0                 | 0.96±0.01^a |
| 15                | 2.79±0.01^b |
| 30                | 2.66±0.11^b |
| 45                | 2.90±0.09^b |
| 60                | 2.81±0.19^b |
| 75                | 2.80±0.16^b |

As shown in Table 7, only temperature of reaction
has a significant effect on the browning intensity shown by values of "Prob > F" less than 0.05. In this study, initial pH and heating time have no significant effect on the browning intensity. The reduced version of the model was performed to improve the model by excluding both insignificant variables. By using the reduced linear model, adjusted $R^2$ increased by about 2.54% (from 80.67 to 83.21). However, the predictive data covered in the model increased by about 10% (from 68.58 to 78.71%). Final equation of factors involved in the reduced linear model is as in Equation (4) and statistical analysis of the reduced model is as shown in Table 9.

Based on the condition suggested by the software (desirability 0.88), the optimum conditions for browning intensity are as follow (1) initial pH 10.92, temperature 140°C, and heating time 37.28 mins; (2) initial pH 11.96, temperature 140°C, and heating time 32.22 mins. The response calculated from the final set of conditions gave the browning intensity of 2.52 at 360 nm.

Table 7. Central composite design for the measure of browning intensity.

| Run | Initial pH | Temperature (°C) | Time (mins) | $A_{360}$ |
|-----|------------|------------------|-------------|-----------|
| 1   | 10         | 130              | 37.5        | 2.39      |
| 2   | 10         | 140              | 30          | 2.54      |
| 3   | 12         | 140              | 45          | 2.44      |
| 4   | 10         | 120              | 30          | 2.01      |
| 5   | 12         | 120              | 30          | 2.02      |
| 6   | 10         | 140              | 45          | 2.48      |
| 7   | 12         | 120              | 45          | 2.13      |
| 8   | 11         | 130              | 37.5        | 2.36      |
| 9   | 10         | 120              | 45          | 2.17      |
| 10  | 11         | 130              | 37.5        | 2.32      |
| 11  | 11         | 140              | 37.5        | 2.43      |
| 12  | 12         | 140              | 30          | 2.59      |
| 13  | 11         | 120              | 37.5        | 2.2       |
| 14  | 12         | 130              | 37.5        | 2.4       |
| 15  | 11         | 130              | 30          | 2.36      |
| 16  | 11         | 130              | 45          | 2.34      |
| 17  | 11         | 130              | 37.5        | 2.39      |

$A_{360} =$ Absorbance at the wavelength of 360 nm.

Table 8. ANOVA for response surface linear model

| Source         | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|----------------|----------------|----|-------------|---------|----------|
| Model          | 0.38042        | 3  | 0.12681     | 23.2555 | < 0.0001 | significant |
| Initial pH     | 0.00001        | 1  | 0.00001     | 0.00183 | 0.9665   |
| Temperature    | 0.38025        | 1  | 0.38025     | 69.73532| < 0.0001 |
| Heating time   | 0.00016        | 1  | 0.00016     | 0.02934 | 0.8666   |
| Residual       | 0.07087        | 13 | 0.00545     |         |          |
| Lack of Fit    | 0.06842        | 11 | 0.00622     | 5.04319 | 0.177    | not significant |
| Pure Error     | 0.00247        | 2  | 0.00123     |         |          |
| Cor Total      | 0.45131        | 16 |             |         |          |

Figure 1 shows the dependency of browning intensity toward the initial pH and temperature at a constant heating time. It is clear that at a constant heating time and initial pH, browning intensity increased moderately with increasing temperature. This may also be seen from Figure 1 where that initial pH in the range of 10-12 has no significant effect on the optimization results. According to Ames and Apriyantono (1994), the rate of Maillard browning increases with pH over the pH range 4-8. When the pH range is extended up to pH 12, the rate of browning in sugar-amino acid model systems shows a maximum at a pH of about 10.

![Figure 1. Three-dimensional diagram of the interactive effects between initial pH and temperature on browning intensity at the constant heating time (37.50 mins)]](image)

Browning intensity $= -0.20753 + 0.0195$ temperature (4)
Table 9. ANOVA for response surface reduced linear model

| Source       | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|--------------|----------------|----|-------------|---------|----------|
| Model        | 0.38025        | 1  | 0.38025     | 80.27133| < 0.0001 |
| Temperature  | 0.38025        | 1  | 0.38025     | 80.27133| < 0.0001 |
| Residual     | 0.07106        | 15 | 0.00474     |         |          |
| Lack of Fit  | 0.06859        | 13 | 0.00528     | 4.27791 | 0.2052   |
| Pure Error   | 0.00247        | 2  | 0.00123     |         |          |
| Cor Total    | 0.45131        | 16 | 80.27133    |         |          |

Table 10. Result of predicted and actual data at optimum condition for browning intensity

| Initial pH | Temperature (°C) | Heating time (min) | A<sub>360</sub> predicted | A<sub>360</sub> actual | Relative error |
|------------|------------------|--------------------|---------------------------|-----------------------|---------------|
| 10.92      | 140              | 37.28              | 2.52                      | 2.64±0.00             | 4.68%         |
| 11.95      | 140              | 32.22              | 2.52                      | 2.67±0.02             | 6.07%         |

A<sub>360</sub> = Absorbance at the wavelength of 360 nm

3.3 Validation model

Validation tests (Table 10) were performed under the optimum condition to determine the adequacy of the model (Equation 4). According to reduced-linear model, the predicted result for browning intensity (2.52) obtained under the optimum conditions was close to the actual response observed. According to Montilha et al. (2017) relative error values in the range, 10-15% is acceptable in an optimization process. From Table 10 the error percentage for the model was less than 7% indicating that the response surface reduced linear model was adequate to predict the browning intensity of Maillard products of the bovine gelatine-xylose system.

4. Conclusion

Optimization using RSM with a central composite design is relevant to obtain the browning intensity of Maillard products of bovine gelatine-xylose model. Based on the validation tests, initial pH of 10.92, reaction temperature of 140°C and heating time of 37.28 minutes were considered as the optimum condition for Maillard reaction of bovine gelatine-xylose with the browning intensity of 2.52 at the wavelength of 360 nm. The linear model is adequate for the response with a coefficient of determination, R<sup>2</sup> value of 0.8429. It means that all parameters have shown a good fit with the experimental data at 95% confidence level.

Acknowledgements

This research was funded by the Indonesia Endowment Fund for Education (LPDP) via Beasiswa Pendidikan Indonesia (PRJ-133/LPDP.3/2017) and RIGS16-332-0496 (IIUM).

References

Ames, J. M. and Apriyantono, A. (1994). Comparison of the non-volatile ethyl acetate-extractable reaction products formed in a xylose-lysine model system heated with and without pH control. Food Chemistry, 50(3), 289–292. https://doi.org/10.1016/0308-8146(94)90135-X

Burin, V.M., Marchand, S., De Revel, G. and Bordignon -Luiz, M.T. (2013). Development and validation of method for heterocyclic compounds in wine: Optimization of HS-SPME conditions applying a response surface methodology. Talanta, 117, 87–93. https://doi.org/10.1016/j.talanta.2013.08.037

Chen, X.-M. and Kitts, D.D. (2008). Antioxidant activity and chemical properties of crude and fractionated maillard reaction products derived from four sugar-amino acid maillard reaction model systems. Annals of the New York Academy of Sciences, 1126(1), 220–224. https://doi.org/10.1196/annals.1433.028

Gu, F., Abbas, S. and Zhang, X. (2009). Optimization of Maillard reaction products from casein – glucose using response surface methodology. LWT - Food Science and Technology, 42(8), 1374–1379. https://doi.org/10.1016/j.lwt.2009.03.012

Hamizah, A., Hammed, A.M., Asiyanihi, H.T.T., Mirghani, M.E.S., Jaswir, I. and Fadzilah, N.H. (2017). Evaluation of catalytic effects of chymotrypsin and Cu<sup>2+</sup> for development of UV-spectroscopic method for gelatine-source differentiation. International Journal of Food Science, 2017, 1–5. https://doi.org/10.1155/2017/2576394

Jaeger, H., Janositz, A. and Knorr, D. (2010). The Maillard reaction and its control during food processing. Pathologie Biologie, 58(3), 207–213. https://doi.org/10.1016/j.patbio.2009.09.016

Kwik, E.J. and Lim, S.I. (2004). The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control. Amino Acids, 27(1), 85–90. https://doi.org/10.1007/s00726-004-0067-7

Liu, Q., Niu, H., Zhao, J., Han, J. and Kong, B. (2016). Effect of the reactant ratio on the characteristics and antioxidant activities of maillard reaction products in xylose model. International Journal of Food Science and Technology, 51, 1–6. https://doi.org/10.1111/ijfs.13342

Ismarti et al. / Food Research 4 (Suppl. 1) (2020) 99 – 106

© 2019 The Authors. Published by Rynnye Lyan Resources
a porcine plasma protein hydrolysate-galactose model system. *International Journal of Food Properties*, 19(1), 99–110. https://doi.org/10.1080/10942912.2015.1017048

Martins, S., Martins, S.I.F.S. and Jongen, W.M.F. (2000). A review of Maillard reaction in food and implications to kinetic modeling. *Trends in Food Science and Technology*, 11(9-10), 364–373. https://doi.org/10.1016/S0924-2244(01)00022-X

Montilha, M., Sbroggio, M.F., Figueiredo, V.R.G., Ida, E.I. and Kurozawa, L.E. (2017). Optimization of enzymatic protein hydrolysis conditions of okara with endopeptidase alcalase. *International Food Research Journal*, 24(3), 1067–1074.

Nursten, H. (2005). The Maillard Reaction. London, UK: The Royal Society of Chemistry.

Rizzi, G. (2011). Chemical structure of colored Maillard reaction products. *Food Reviews International*, 13(1), 1–28. https://doi.org/10.1080/87559129709541096

Tan, T.C., Alkarkhi, A.F.M. and Easa, A.M. (2012). Assessment of the ribose-induced Maillard reaction as a means of gelatine powder identification and quality control. *Food Chemistry*, 134(4), 2430–2436. https://doi.org/10.1016/j.foodchem.2012.04.049

Van Boekel, M.A.J.S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances*, 24(2), 230–233. https://doi.org/10.1016/j.biotechadv.2005.11.004

Wang, H.Y., Qian, H. and Yao, W.R. (2011). Melanoidins produced by the Maillard reaction: Structure and biological activity. *Food Chemistry*, 128(3), 573–584. https://doi.org/10.1016/j.foodchem.2011.03.075

Zhang, K., Zhang, B., Chen, B. and Jing, L. (2016). Modeling and optimization of Newfoundland shrimp waste hydrolysis for microbial growth using response surface methodology and artificial neural network, *Marine Pollution Bulletin*, 109(1), 245-252. https://doi.org/10.1016/j.marpolbul.2016.05.075