Food Research 3 (5) : 525 - 529 (October 2019)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Optimisation of browning index of Maillard reaction in gelatine powder by response surface methodology (RSM) for halal authentication

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Article history:
Received: 5 March 2019
Received in revised form: 3 April 2019
Accepted: 5 April 2019
Available Online: 12 April 2019

Keywords:
Gelatine,
Halal,
Maillard Reaction,
Traceability,
UV-Vis Spectroscopy

DOI:
https://doi.org/10.26656/fr.2017.3(5).116

Abstract

Gelatine, as the product of collagen extraction from animals, is widely used in the food industry. In a glance, the physical properties of gelatine from several sources such as fish, bovine and porcine are similar. Therefore, distinguishing between the sources of gelatine is a tedious task. The differentiation of the gelatine from its sources requires an approach of a chemical reaction. This paper focused on the optimisation of Maillard reaction from different sources of gelatine by Response surface methodology (RSM). The experiment was designed with several imperative parameters; temperature, time and presence of metal ion Cu^{2+}. The response was recorded from the absorbance of reacted gelatine mixture at specific wavenumber (420 nm) through UV-Vis instrumentation. The optimal reaction condition of all type of gelatines in water bath was 95°C for 9 hrs. From solution given, only 5 mM concentration of metal ion Cu^{2+} has an influence on the bovine gelatine compared to fish and porcine gelatine. Maillard reaction with a combination of UV-Vis spectroscopy is one of the convenient protocols for rapid authentication purpose. RSM help to optimize the reaction condition of gelatine from different sources.

1. Introduction

The halal authentication on gelatine as food ingredient hitches concern and demand worldwide as the number of Muslim approaching 2 billion. The reason is gelatine can only be derived from animals while exposed to a higher chance of adulteration which brings the status of *haram* (prohibited) and syubhah (ambiguous). In the case of gelatine from porcine no doubt that it is forbidden for human consumption along with the case of gelatine from bovine without proper slaughtering in compliance with Islamic teaching (Fadzillah *et al*., 2011). Moreover, the *Istihalah* (transformation) process in gelatine from animal's collagen is not acceptable as the alteration is incomplete where no chemical changes occur (Jamaludin *et al*., 2011). According to Grand View Research, about 40.6% of the raw material of gelatine in 2015 is from pork skin which becomes major usage in the market volume. Meanwhile, gelatine from bovine is expecting to grow fast approaching 2024 at a compound annual growth rate (CAGR) of 4.9%.

Scientifically, gelatine is a high molecular weight polypeptide of partial collagen hydrolysis from animals mainly mammal's hides, bones and skins. Acids and alkaline treatments are being used on the animal's connective tissue and skins to transform the collagen structure helical structure into random coils (Nhari *et al*., 2012). The acidic treatment is known as gelatine Type A which applicable for porcine skin to avoid saponification of higher fat content on the skin. Fish skin also undergoes mild acidic treatment for gelatine extraction from both cold and warm-water fish species (Gómez-Guillén *et al*., 2009). Meanwhile, Type B gelatine represents alkaline treatment to the animal tissue that has a lesser degree of covalent bonding in collagen as bovine skin.

The building blocks of gelatine are known as amino acids. These amino acids can undergo Maillard reaction when heated with reducing sugar and form a brown

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eISSN: 2550-2166 /

fluorescence product. Maillard reaction causes brown colour development by three steps as shown in Figure 1. The first step involves the rearrangement of sugar into N -glycosylamine after condensation with the amino group to form the formation of Amadori product from aldose sugar or Heyns product from ketose sugar. The second step is the sugar fragmentations that cause the amino group to be released and Strecker degradation which creates aldehydes and many essential intermediate compounds. Further dehydration, fragmentation, cyclization and polymerisation in the last step take place by carbonyl compound will result in the formation of the brown pigments known as melanoidins (Bastos *et al*., 2012).

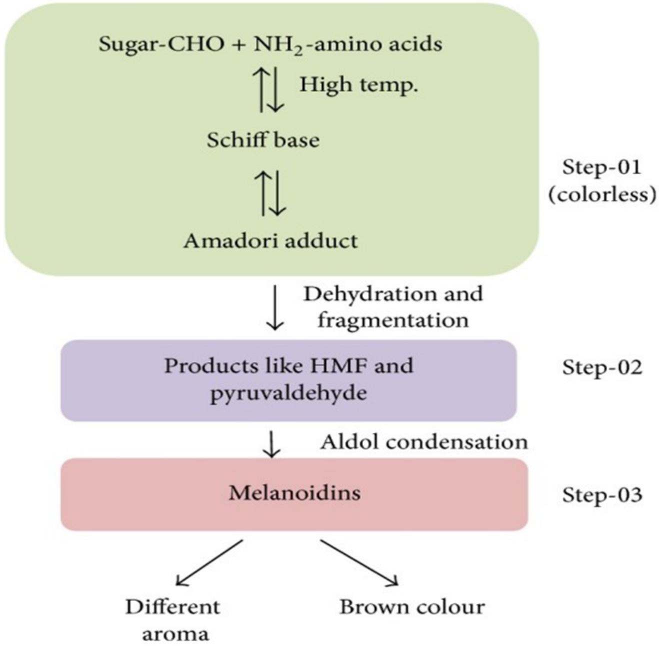

Figure 1. Schematic representation of Maillard reaction and colour formation in food (Tamanna and Mahmood, 2015).

The amino acid profiling of gelatine varies with their origins (Azilawati *et al*., 2015). Thus, during Millard reaction, amino acids in gelatine contribute differently toward colour development; the degree of browning contribution can be investigated by UV-Vis spectroscopy. Besides, the browning was accelerated by the presence of metal ions such as Fe^{2+} and Cu^{2+} but inhibited by the presence of NaCl (Kwak and Lim, 2004). The previous study by Tan *et al*. (2012) explained the extent of browning during the Maillard reaction of gelatine from bovine or porcine at 110°C for 24 hrs. However, there was no report of the interaction between the factors affecting on Maillard reaction of gelatine such as the temperature, time, the presence and concentration of inorganic compounds. The parameters of these several factors are difficult to control individually and may have some symbiosis effect.

Response surface methodology (RSM) is a useful tool to investigate the optimum conditions of Maillard reaction model system (Gu *et al*., 2009; Arachchi *et al*., 2017). RSM can improve and optimize complex processes. Moreover, RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions with acceptable collective statistical results from sufficient information (Yang *et al*., 2012). Thus, this paper focused on the optimisation of rapid color development of fish, bovine and porcine gelatine during Maillard by response surface methodology (RSM) for halal authentication purpose.

2. Material and methods

2.1 Sample preparation for Maillard reaction of gelatine from different sources

0.5 M of D-(+)-xylose was mixed accordingly to 2% of fish, bovine and porcine gelatine solutions (Sigma Co., USA). The colourless solutions were heated in a water bath until each mixture changed into brown solutions. Another set of experiments was prepared and catalysed with copper chloride ($CuCl_2$). The solutions were cooled in an ice bath to prevent further reaction. About 200 µL of the sample was taken out in triplication and filled into 96-well plate to measure the absorbance at 420 nm using UV-spectroscopic (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). The heated gelatine and xylose mixtures were termed as MRPs and the absorbance reading was referred to as browning index measurement. The procedure to carry on Maillard reaction was followed from Kwak and Lim (2004) with some modification.

2.2 Experimental design for optimisation of Maillard reaction on gelatine by response surface methodology (RSM)

RSM was used after the one-factor-at-a-time study conducted previously by Hamizah *et al*. (2017), to identify the optimum conditions by using Stat-Ease Design-Expert® version 11.0.1.0 (USA). A central composite experimental design (CCD) was generated on the factors of temperature (X_1), time (X_2) and concentration of metal ion Cu^{2+}(X_3). One categorical factor was included at three types of gelatine: fish, bovine and porcine which led to 60 sets of experiments. The absorbance represented the response after the Maillard reaction of gelatine with xylose is completed. Three coded levels for three numerical factors were set as in Table 1 with three replications for each run. The coded levels used for each independent variable are -1, 0 and 1.

Table 1. The factors and code levels in RSM

Factor	Units	Code Levels		
		-1	0	1
Temperature (X_1)	°C	35	65	95
Time (X_2)	Hour	3	6	9
Concentration of Cu^{2+} ion (X_3)	mM	0	2.5	5

2.3 Statistical analysis

Analysis of variance (ANOVA), standard deviations and other statistical calculations (at P>0.05) were performed and analysed.

3. Result and discussion

Based on previous data of one-factor-at-time experiments, temperature and time found to be directly proportional to the absorbance of the gelatine-xylose mixture after undergoing Maillard reaction from 6 to 24 hrs at 95°C. Throughout that duration, slow and fast reaction phase was detected after 6 hrs of the reaction. About 2 mM of a metal ion from $CuCl_2$ added to react as a catalyst to speed up the Maillard reaction (Hamizah *et al*., 2017).

Table 2 shows that the highest value of absorbance was obtained from the highest temperature. In specific, the highest absorbance value for fish gelatine is at run 21, bovine gelatine at run 59 and porcine gelatine at run 28 respectively. However, the trend of time and concentration of metal ion Cu^{2+} were irregular. The presence of metal ion and lower time cause decreased in the absorbance of fish gelatine but enhance the reaction time for bovine and porcine gelatine. According to Kwak and Lim (2004), the effect of metal ion on browning is also depending on the type of amino acid where in this case vary among the type of gelatine.

Statistical analysis of experimental design was carried out by response surface methodology (RSM) to study the interaction of all factors. Analysis of variance (ANOVA) was performed to calculate the regression coefficients of the coded equation and verify the solution from RSM. The models present values for the coefficients of determination, R^2 as 0.94 and coefficients of variation, CV as 11.12%. The high R^2 and low CV indicate a good precision and reliability of the experiment as showing less variation which relative to its mean. The F-value and probability > F value was used to determine the significance of each coefficient. Table 3 shows that the model was significant (P<0.05). The linear effect and interaction of temperature (X_1) and time (X_2) were significant and positively related to the browning index. The interaction of time (X_2) and concentration of metal ion Cu^{2+} (X_3) were also significant, although the concentration of metal ion Cu^{2+} (X_3) was negatively related to browning index. The fitted model equations are shown as in Equation (1), Equation (2) and Equation (3) for fish, bovine and porcine gelatine respectively.

$$\ln(\text{Absorbance})_{\text{Fish}} = -1.31240 - 0.07462X_1 - 0.02934X_2 + 0.04132X_3 + 0.00135X_1X_2 + 0.00008X_1X_3 - 0.01325X_2X_3 + 0.000713X_1^2 + 0.00421X_2^2 + 0.00294X_3^2 \quad (1)$$

$$\ln(\text{Absorbance})_{\text{Bovine}} = -0.56222 - 0.07462X_1 - 0.07759X_2 + 0.11633X_3 + 0.00135X_1X_2 + 0.00008X_1X_3 - 0.01325X_2X_3 + 0.000713023X_1^2 + 0.004166391X_2^2 + 0.002881263X_3^2 \quad (2)$$

$$\ln(\text{Absorbance})_{\text{Porcine}} = -1.48134 - 0.06757X_1 - 0.05662X_2 + 0.05020X_3 + 0.00135X_1X_2 + 0.00008X_1X_3 - 0.01325X_2X_3 + 0.000713023X_1^2 + 0.004166391X_2^2 + 0.002881263X_3^2 \quad (3)$$

The difference between Predicted R^2 (0.83) and Adjusted R^2 (0.92) is less than 0.2 showing a reasonable agreement. The value of Adequate Precision (20.77) showed an adequate signal which suggests that this model can be used to navigate the design space. Figure 2 shows the perturbation model graphs of absorbance over all factors affecting the Maillard reaction of gelatine. The temperature labelled as X_1 was directly proportional to the absorbance showing that the most influencing factors

Table 2. Five highest response dependent variables of absorbance from the composite experimental design matrix for three types of gelatine subjected to Maillard reaction

Run	X_1: Temperature (°C)	X_2: Time (hour)	X_3: Concentration of Cu^{2+} ion (mM)	X_4: Type of Gelatine	Absorbance
57	95	3	5	Fish	0.1702±0.0100
43	95	3	0	Fish	0.2005±0.0199
34	95	6	2.5	Fish	0.2556±0.0139
17	95	9	5	Fish	0.3661±0.0105
21	95	9	0	Fish	0.5922±0.0272
11	95	6	2.5	Bovine	0.2532±0.0120
41	95	3	0	Bovine	0.2876±0.0310
8	95	9	5	Bovine	0.7631±0.0467
15	95	9	0	Bovine	0.8072±0.0454
59	95	3	5	Bovine	1.1740±0.1119
52	95	3	5	Porcine	0.2880±0.0229
49	95	3	0	Porcine	0.2905±0.0255
25	95	9	5	Porcine	0.3560±0.0217
13	95	9	0	Porcine	0.6177±0.0314
28	95	6	2.5	Porcine	0.7901±0.0851

The data represent mean ± standard deviation of three replicates.

eISSN: 2550-2166

in Maillard reaction of gelatine. Meanwhile, the factor of time, X_2 and concentration of metal ion Cu^{2+}, X_3 were lower indicating less important for the reaction.

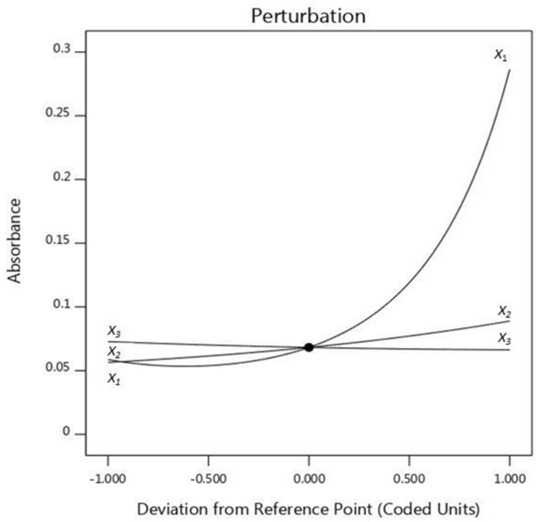

Figure 2. Perturbation graph over all types of gelatine on three factors affecting the Maillard Reaction

The software provided solutions for optimum reaction conditions. Table 4 shows the solution selected from post analysis of response surface methodology for each type of gelatine for all factors influencing Maillard reaction. The values suggested can be used to validate the model. As can be seen from the results, the error obtained is less than 5% which shows the reliability and the applicability of the model. From the results, fish and porcine gelatine displayed increment in browning during 9 hrs without the presence of metal ion compared to bovine gelatine. Time and temperature have a more significant effect on browning compared to the concentration of Cu^{2+}. This means that the concentration of Cu^{2+} could be fixed at the lower level as a catalyst will speed up the reaction at a low concentration (Kwak and Lim, 2004).

The Maillard reaction products (MRPs) made from sugar–tuna stomach hydrolysate model system was tested at 95°C and 115°C in phosphate buffer (Sumaya-Martinez *et al*., 2005), which is comparable to the data obtained in this study. Moreover, in Maillard reaction system, peptides cross-linked rapidly when the temperature reached 110-120°C when testing soybean peptides where the increase of macromolecule products was also accompanied by severe browning and pH decrement. It can be concluded that temperature is an important factor for the reaction and correlate to both thermal degradation and Maillard reaction. Maillard reaction decreased the content of bitter amino acid and peptides below 1000 Da thus reduced the bitter properties of the final products (Lan *et al*., 2010).

4. Conclusion

Response surface methodology (RSM) was utilised in optimising the variables of Maillard reaction of gelatine from fish, bovine and porcine. The optimum browning index from absorbance reading of fish and porcine gelatine was achievable at the highest temperature and time without the presence of metal ion compared to bovine gelatine. These optimisation results are very useful for further targeted discriminate analysis

Table 3. Variance analysis for optimisation of Maillard reaction with all type of gelatine

Source	Sum of	Df	Mean	F-value	p-value	
Model	35.73229	17	2.1019	38.64948	< 0.0001	Significant
X_1-Temperature	22.33515	1	22.33515	410.696	< 0.0001	
X_2-Time	0.688357	1	0.688357	12.65743	0.000943	
X_3-Concentration of Cu^{2+} ion	0.016876	1	0.016876	0.310312	0.580446	
X_4-Type of Gelatine	4.613567	2	2.306784	42.41686	< 0.0001	
X_1X_2	0.3537	1	0.3537	6.503792	0.014501	
X_1X_3	0.000813	1	0.000813	0.014945	0.903284	
X_1X_4	0.298732	2	0.149366	2.746522	0.075684	
X_2X_3	0.237181	1	0.237181	4.361263	0.042866	
X_2X_4	0.105347	2	0.052674	0.968556	0.387948	
X_3X_4	0.21	2	0.105	1.930724	0.1577	
X_1^2	3.401128	1	3.401128	62.53954	< 0.0001	
X_2^2	0.011819	1	0.011819	0.217331	0.64349	
X_3^2	0.002781	1	0.002781	0.05114	0.822189	
Residual	2.284113	42	0.054384			
Lack of Fit	2.274617	27	0.084245	133.0788	< 0.0001	Significant
Pure Error	0.009496	15	0.000633			
Cor Total	38.01641	59				
Pure Error	35.73229	17	2.1019	38.64948	< 0.0001	
Cor Total	22.33515	1	22.33515	410.696	< 0.0001	

Table 4.Three selected solution for optimisation of Maillard reaction for different types of gelatine

Type of Gelatine	Temperature (°C)	Time (hour)	Concentration of Cu^{2+} ion (mM)	% Error of validation
Fish	95	9	0	+ 2.72
Bovine	95	9	5	+ 3.15
Porcine	95	9	0	+ 3.15

of gelatine. It was also helpful to verify the research finding of one-factor-at-time and understanding the interaction between all factors.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was financially supported under the Fundamental Research Grant Scheme (FRGS/1/2016/STG01/UIAM/02/5) with special thanks to the Malaysia Ministry of Education.

References

Arachchi, S.J.T., Kim, Y.-J., Kim, D.-W., Oh, S.-C. and Lee, Y.-B. (2017). Optimization of Maillard reaction in model system of glucosamine and cysteine using response surface methodology. *Preventive Nutrition and Food Science,* 22(1), 37-44. https://doi.org/10.3746/pnf.2017.22.1.37

Azilawati, M. I., Hashim, D.M., Jamilah, B. and Amin, I. (2015). RP-HPLC method using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate incorporated with normalization technique in principal component analysis to differentiate the bovine, porcine and fish gelatins. *Food Chemistry*, 172, 368-376. https://doi.org/10.1016/j.foodchem.2014.09.093

Bastos, D.M., Monaro, E., Siguemoto, E. and Sefora, M. (2012). Maillard Reaction Products in Processed Food: Pros and Cons. InTech. Retrieved from InTechOpen website: https://www.intechopen.com/books/food-industrial-processes-methods-and-equipment/maillard-reaction-products-in-processed-food-pros-and-cons. https://doi.org/10.5772/31925

Fadzillah, N.A., Man, Y.B.C., Jamaluddin, M.A., Rahman, S.A. and Al-Kahtani, H.A. (2011). Halal food issues from Islamic and modern science perspectives. *2nd International Conference on Humanities, Historical and Social Sciences*, 171, 59–163.

Gu, F.-I., Abbas, S. and Zhang, X.-M. (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

Gómez-Guillén, M., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E., Giménez, B. and Montero, P. (2009). Fish gelatine: a renewable material for developing active biodegradable films. *Trends in Food Science and Technology*, 20(1), 3-16. https://doi.org/10.1016/j.tifs.2008.10.002

Hamizah, A., Hammed, A.M., Asiyanbi-H, T.T., Mirghani, M.E.S., Jaswir, I. and Fadzillah, N.A. (2017). Evaluation of Catalytic Effects of Chymotrypsin and Cu^{2+} 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

Jamaludin, M.A., Zaki, N.N.M., Ramli, M.A., Hashim, D.M. and Rahman, S.A. (2011). Istihalah: Analysis on The Utilization of Gelatin in Food Products. *2nd International Conference on Humanities, Historical and Social Sciences,* 17, 174-178.

Kwak, 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

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., Xia, W., Tian, H. and Xiao, Z. (2010). Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose–soybean peptide system: Further insights into thermal degradation and cross-linking. *Food Chemistry*, 120 (4), 967-972. https://doi.org/10.1016/j.foodchem.2009.11.033

Nhari, R.M.H.R., Ismail, A. and Che Man, Y.B. (2012). Analytical methods for gelatine differentiation from bovine and porcine origins and food products. *Journal of Food Science*, 77(1), R42-R46. https://doi.org/10.1111/j.1750-3841.2011.02514.x

Sumaya-Martinez, M.T., Thomas, S., Linard, B., Binet, A. and Guerard, F. (2005). Effect of Maillard reaction conditions on browning and antiradical activity of sugar–tuna stomach hydrolysate model system. *Food Research International*, 38(8-9), 1045-1050. https://doi.org/10.1016/j.foodres.2005.03.015

Tamanna, N. and Mahmood, N. (2015). Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *International Journal of Food Science,* 2015, 526762. http://dx.doi.org/10.1155/2015/526762

Tan, T., Alkarkhi, A.F.M. and Mat, A. (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

Yang, C., Song, H.L. and Chen, F. (2012). Response surface methodology for meat-like odorants fom Maillard reaction with glutathione I: the optimization analysis and the general pathway exploration. *Journal of Food Science*, 77(9), C966-C974. https://doi.org/10.1111/j.1750-3841.2012.02863.x.