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FULL PAPER

Discrimination of porcine and bovine gelatines based on reducing sugar types on Maillard reaction

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Abstract

Increasing awareness among Muslims with respect to the Halal status of food has intensified efficient and effective food-source authentications that are science-based-protocol. Traceability of gelatine to its source remains a daunting scientific task prior to classification of gelatine Halal status. The methodology of this study involves UV-Vis spectroscopic measurement of the degree of browning during Maillard reaction of different gelatine sources with the different type of reducing sugar. D-(+)-xylose has the highest value of browning value (A_{420nm}) of Maillard reaction with gelatine among compared to another type of reducing sugar used. Principle Component Analysis (PCA) used to differentiate the sources of gelatine and successfully identify the major contribution of D-(+)-xylose in the browning value. The score plots of the first principle component (PC1) and the second component (PC2) could be classified and differentiated of gelatine types (porcine, bovine and fish) using variables of browning value of Maillard reaction of gelatinees with different types of sugar, as evaluated using spectrophotometer at 420 nm.

1. Introduction

Industrialists and consumers required that halal assessment of animal products including meat and gelatines must be objective and was supported by scientific research (Hameed *et al*., 2018). This has attracted for the development of halal science in order to investigate and to provide scientific proves regarding the halalness status of different sources of food raw materials and ingredients, especially gelatine (Mutalib *et al*., 2015). Gelatine is taken into account as frequently object studies in halal food ingredient due to its application in food and pharmaceutical products. Gelatine comes from collagen hydrolysis with specific physico-chemical properties with several functions including as gelling agent, foaming agent, thickener, and binding agent. The potential market of gelatines has increased significantly today (Malik *et al*., 2015).

Gelatine and gelatine-based products are currently classified as doubtful because gelatine from haram (porcine) source is abundantly used in food systems. It is used as ingredients in various food products but become difficult to be distinguished since it is colorless, odorless and tasteless (Sultana *et al*., 2018). Therefore, the halal authentication of gelatine catches more concern and attention among halal scientist (Raraswati *et al*., 2013). Some review articles describing the authentication of gelatine using different methods have existed for analysis of gelatine sources (Hameed *et al*., 2018). Such methods are real-time PCR for analysis of deoxyribonucleic acid or DNA (Sudjadi *et al*., 2015), high-performance liquid chromatography (HPLC) (Raraswati *et al*., 2013), liquid chromatography-mass spectrometry (LC-MS) (Grundy *et al*., 2015), electrophoresis (Malik *et al*., 2015), and enzyme-linked immune-sorbent assays (Nur Azira *et al*., 2016). These methods are very complex and need sophisticated instruments, therefore, simple methods based on spectroscopic methods such as ultraviolet-visible (UV-vis) and Fourier transform infrared (FTIR) spectroscopy via analysis of specific components in gelatine samples should be developed.

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Like other molecular spectroscopies used for gelatines differentiation (Hashim *et al*., 2015; Hermanto *et al*., 2015), UV-Vis spectroscopy can serve as a straightforward method for halal authentication of gelatine. Many UV-Vis spectroscopic analytical procedures have been found useful for food analyses such as detection of melamine in fish and phenols compound in olive oils (Munjanja and Sanganyado, 2015). Recently, browning value from melanoidins that formed in Maillard systems has been widely used as an indicator of reaction progress (Etxabide *et al*., 2015). The absorption of the browning compound from Maillard reaction products can be measured at wavelength 420 nm as single spectrum. Some researchers also consider a correction for any turbidity in the samples by measuring the absorption at 550 nm (Tan *et al*., 2012).

Maillard reaction is known to give browning color to the food because of the high molecular weight of polymers containing furan ring and nitrogen (Tamanna and Mahmood, 2015). This reaction involves the interaction between the amino compound and reducing sugar and it is responsible for non-enzymatic browning color in cooked food. The color development in Maillard reaction has three stages (Bastos *et al*., 2012). Firstly, the reducing sugar undergoes condensation with amino group and cause the formation of Amadori product from aldose sugar or Heyns product, from ketose sugar that rearranged into N-glycosylamine. Next, the amino group is released by the sugar fragmentation and Strecker degradation which create aldehydes and many essential intermediate compounds. Finally, further dehydration, fragmentation, cyclization and polymerization were taken place by carbonyl compound resulted in melanoidins, the brown pigments.

During the Maillard reaction, amino acids contribute differently toward color development (Natella *et al*., 2002). The degree of color contribution was investigated using UV-spectroscopy. The amino acid profiles of gelatine varied with their origin (Azilawati *et al*., 2015), therefore, it is possible that degree of color development by UV-Vis spectroscopy will vary when it was subjected to Maillard reaction. This variation combined with chemometrics techniques such as Principal component analysis (PCA) can be related to the source of gelatine (Rufian-Henares and Morales, 2007). PCA is one of chemometrics techniques that have been used as a tool to analyse the numerous amount of complex data generated from analytical instruments (Hassan *et al*., 2018). As one of multivariate data analysis, PCA uses the application of optimal mathematical and statistical methods to process data (Roussel *et al*., 2014). PCA is an unsupervised pattern recognition technique used to make the differentiation and classification among objects including gelatine sources. PCA protrudes the original data in reduced dimensions described by the principle components (PCs) as PC1 and PC2 (Rohman *et al*., 2012). This study focused on the effects of reducing sugar types on the rapid color development of Maillard reaction to distinguish the sources of gelatines from fish, bovine and porcine. The effort has been made to develop a rapid and less expensive method that based on the browning values after Maillard reaction of gelatine by UV-Vis spectroscopy.

2. Material and methods

D-(+)-glucose, D-(+)-galactose, D-(+)-xylose, D-(+)-mannose, D-(-)-fructose, D-(+)-maltose monohydrate, L-rhamnose monohydrate, L-arabinose, porcine gelatine, and bovine gelatine were purchased from Sigma Co. (Aldrich, USA). Fish gelatine was laboratory prepared according to (Fan *et al*., 2017).

2.1 Maillard reaction of gelatine with different types of reducing sugar

An about 0.5 M of D-(+)-glucose, D-(+)-galactose, D-(+)-xylose, D-(+)-mannose, D-(-)-fructose, D-(+)-maltose monohydrate, L-rhamnose monohydrate and L-arabinose (Sigma Co., USA) solutions and 3 mL of 2% of gelatine solutions from fish, bovine or porcine were heated at temperature 95^{o}C for 9 hrs. The effect of different types of sugar on the browning index of the Maillard reaction of different gelatine sources was observed within 3, 6, and 9 hrs intervals. The solution was cooled in ice at -4^{o}C and directly used for browning measurement (Kwak and Lim, 2004).

2.2 Browning value from UV-Vis spectroscopy

Browning value of different of reducing sugar on Maillard reaction of different gelatine sources (fish, bovine and porcine) was determined using UV-spectroscopic (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer) at wavelength 420 nm. About 200 µL of sample triplicated into 96-well plate to measure their absorbance with gelatine solution from each source as blank. When necessary, appropriate dilutions made to have an optical density of less than 1.5 (Tamana and Mahnood, 2015).

2.3 Principle component analysis (PCA)

The browning *value* of Maillard reaction of gelatine with all type of sugar was analysed in PCA by using Unscrambler 9.7 (Camo, USA) software. The type of gelatine was set as the variables, whereas the browning value from Maillard reaction of D-(+)-glucose, D-(+)-galactose, D-(+)-xylose, D-(+)-mannose, D-(-)-fructose,

D-(+)-maltose monohydrate, L-rhamnose monohydrate and L-arabinose at wavelength 420 nm of UV-Vis spectroscopy were used as input data (Nur Azira *et al*., 2012).

2.4 Statistical analysis

Principal component analysis for classification of objects was analysed using Unscrambler® software (CAMO software).

3. Result and discussion

Figure 1 shows the absorbance values of different sugar types at wavelength of 420 nm, which also known as browning value. The reaction was controlled in water bath at 95°C in between three-time intervals. The Maillard reaction was known to take place within the reducing sugar and amino group at high temperature. The browning value showed similar result and increased with reaction time for all types of sugar. However, the fish gelatine solution without sugar remains unchanged due to the absence of electron-donating group to proceed with Maillard reaction. After 9 hrs, the Maillard reaction of fish gelatine with D-(+)-xylose has the highest browning value at 0.248, followed by D-(-)-fructose and D-(+)-galactose. The monosaccharide, a simple sugar, is more reactive than disaccharide like D-(+)-maltose monohydrate. While in monosaccharide, pentose sugars such as D-(+)-xylose,D-(-)-fructose,L-arabinose and L-rhamnose monohydrate are reported to be more reactive than hexoses, for instance D-(+)-glucose, D-(+)-mannose and D-(+)-galactose (Lund and Ray, 2017).

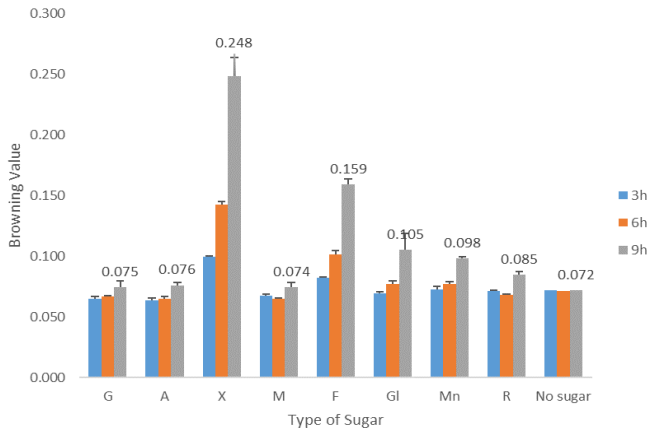

Figure 1. Browning value of Maillard reaction of fish gelatine with different types of sugar at 420 nm. (G: D-(+)-glucose, A: L-arabinose, X: D-(+)-xylose, M: D-(+)-maltose monohydrate, F: D-(-)-fructose, Gl: D-(+)-galactose, Mn: D-(+)-mannose, and R: L-rhamnose monohydrate).

The solution of bovine gelatine in the absence of reducing sugar already has a high browning value as shown in Figure 2. This is due to darker colour of the raw materials in bovine gelatine compared to other sources of gelatine (Tan *et al*., 2012; Chuaynukul *et al*., 2017). Therefore, only D-(+)-xylose, D-(-)-fructose, D-(+)-galactose and D-(+)-mannose have impact on the browning value of Maillard reaction. The additional of L-arabinose and D-(+)-maltose monohydrate, in contrast, make the gelatine solution more diluted. D-(+)-galactose found out to has high browning value although D-(+)-galactose and D-(+)-glucose are both hexoses. It is reported to be more reactive due to the reactive open-chain form in a higher steady-state concentration (Liu *et al*., 2016). In addition, as seen in fish gelatine, Maillard reaction of bovine gelatine remarkably accelerate to 0.420 with D-(+)-xylose starting from 3 hrs to 9 hrs.

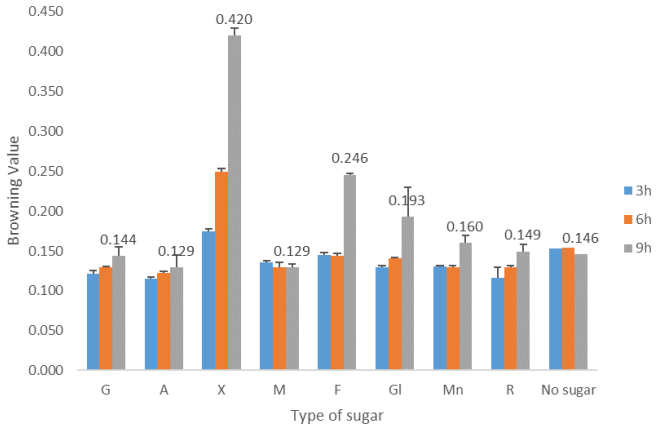

Figure 2. Browning value of Maillard reaction of bovine gelatine with different types of sugar at 420 nm. (G: D-(+)-glucose, A: L-arabinose, X: D-(+)-xylose, M: D-(+)-maltose monohydrate, F: D-(-)-fructose, Gl: D-(+)-galactose, Mn: D-(+)-mannose, and R: L-rhamnose monohydrate)

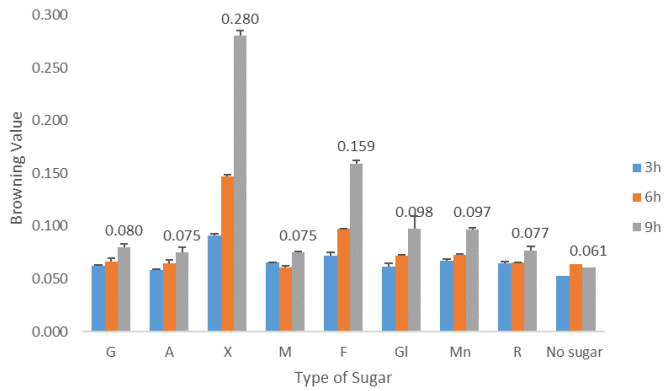

Figure 3. Browning value of Maillard reaction of porcine gelatine with different types of sugar at 420 nm. (G: D-(+)-glucose, A: L-arabinose, X: D-(+)-xylose, M: D-(+)-maltose monohydrate, F: D-(-)-fructose, Gl: D-(+)-galactose, Mn: D-(+)-mannose, and R: L-rhamnose monohydrate).

In high temperature, porcine gelatine successfully undergoes Maillard reaction with all type of sugar after 9 hrs. Other study showed the same observation in porcine

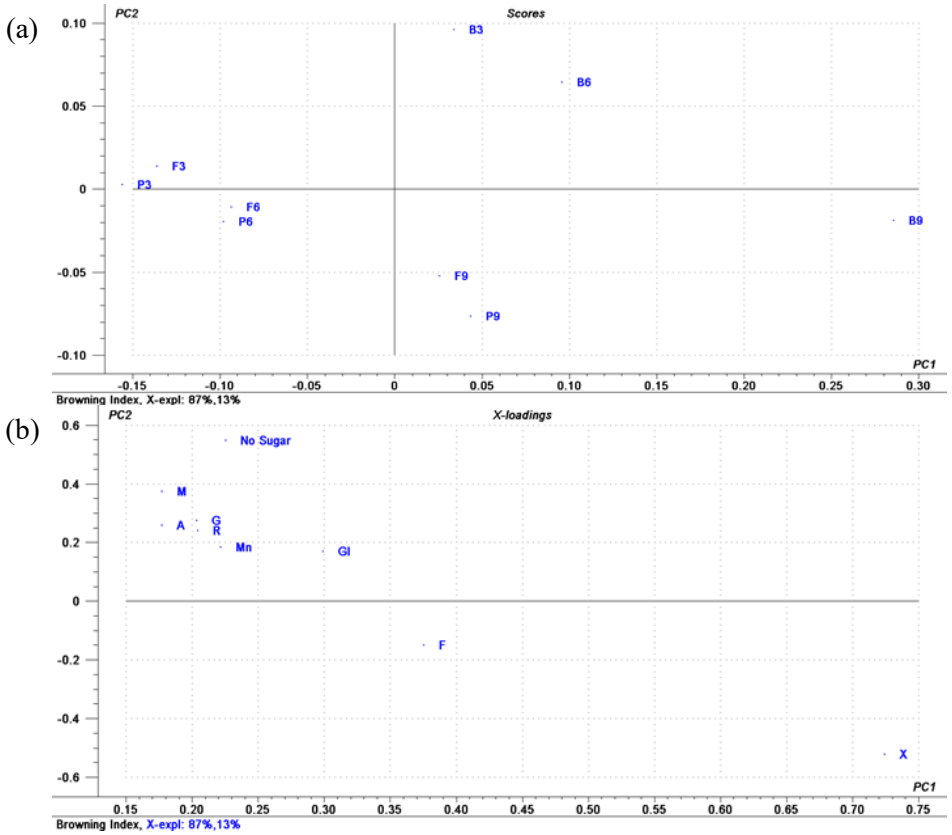

Figure 4. The a) score plot and b) loading plot of browning value of all type of reducing sugar with fish, bovine and porcine gelatine.

plasma protein hydrolysate-galactose model system of Maillard reaction as the heating time increased (Liu *et al*., 2016). This is could be explained that reaction takes place fast at higher temperature. The browning value of porcine gelatine solution without sugar in Figure 3 is the lowest compared to all solution with sugar content. The most substantial increment of browning values is D-(+)-xylose, D-(-)-fructose, D-(+)-galactose which is consistent with the reaction from fish and bovine gelatine at regular intervals.

The acquired value of browning value from Maillard reaction of gelatine from different sources with D-(+)-glucose, D-(+)-galactose, D-(+)-xylose, D-(+)-mannose, D-(-)-fructose, D-(+)-maltose monohydrate, L-rhamnose monohydrate and L-arabinose were analysed by PCA as shown in Figure 4 for the differentiation of gelatine sources. The first (PC1) and second (PC2) component account for 87% and 13% of the variation in the browning value of Maillard reaction at 420 nm wavelength, respectively. The score plot presented the separation of fish, bovine and porcine gelatine. The positive side of PC1 and PC2 were successfully dominated by bovine gelatine, while the negative side of both PCs was subjected for fish and porcine gelatine. The distance of porcine and fish gelatine was adjacent to each other parallel to the browning value due to the same nature of collagen pre-treatment during gelatine extraction (Nur Azira *et al*., 2012). Based on this, the score plots of PC1 and PC2 could be classified and differentiated of gelatine types (porcine, bovine and fish) using variables of browning value of Maillard reaction of gelatines with different types of sugar, as evaluated using spectrophotometer at 420 nm. In addition, the loading plot showed the contribution of variables from reducing sugar to the separation of gelatine sources (Tan *et al*., 2012). D-(+)-xylose has the biggest contribution in the PCA model as it is far away from the point of reaction condition without sugar as the control of experiment.

4. Conclusion

The types of reducing sugar involved in Maillard reaction of gelatine have a significant effect on the brown color development. The browning value of absorbance at 420nm revealed the consequence of sugar from D-(+)-xylose > D-(-)-fructose >D-(+)-galactose consistently with all type of gelatine. Principle Component Analysis (CA) of the browning value also showed the outstanding influence of D-(+)-xylose from other sugar in Maillard reaction to assist the separation of gelatine from fish, bovine and porcine. In term of gelatine from different sources, bovine is successfully remarkable from other types of gelatine. Porcine and fish however showed similarity which requires further analysis for a distinctive separation. The sources of gelatine could be differentiated based on rapid colour development of Maillard reaction in combination with PCA. The score plots of first principle component (PC1) and second component (PC2) could be classified and

differentiated of gelatine types (porcine, bovine and fish) using variables of browning value of Maillard reaction of gelatines with different types of sugar, as evaluated using spectrophotometer at 420 nm. The further study on sugar loss and amino acids lost during Maillard reaction will supervise a better understanding of the separation of gelatine sources. This finding can be developed into a simple protocol of gelatine authentication that will specifically benefit the Halal food industry and Muslim consumers all over the world.

Conflict of Interest

The authors declare no conflict of interest.

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References

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 principle component analysis to differentiate the bovine, porcine and fish gelatines. *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. In Valdez, B. (Ed.). Food Industrial Processes. InTechOpen [Online E-book]. https://doi.org/10.5772/31925

Chuaynukul, K., Nagarajan, M., Prodpran, T., Benjakul, S. and Prasarpran, S. (2017). Impacts of plasticizer and pre-heating conditions on properties of bovine and fish gelatine films fabricated by thermo-compression molding technique. *Italian Journal of Food Science,* 29(3), 487–504.

Etxabide, A., Uranga, J., Guerrero, P. and Caba, K.D. (2015). Improvement of barrier properties of fish gelatine films promoted by gelatine glycation with lactose at high temperatures. *LWT - Food Science and Technology,* 63(1), 315-321. https://doi.org/10.1016/j.lwt.2015.03.079

Fan, H., Dumont, M.J. and Simpson, B.K. (2017). Extraction of gelatine from salmon (*Salmo salar*) fish skin using trypsin-aided process: optimization by Plackett-Burman and response surface methodological approaches. *Journal of Food Science and Technology*, 54(12), 4000-4008. https://doi.org/10.1007/s13197-017-2864-5

Grundy, H.H., Reece, P., Buckley, M., Solazzo, C.M., Dowle, A.A. and Ashford, D. (2016). A mass spectrometry method for the determination of the species of origin of gelatine in foods and pharmaceutical products. *Food Chemistry,* 190, 276–84. https://doi.org/10.1016/j.foodchem.2015.05.054

Hameed, A.M., Asiyanbi, T., Idris, M., Fadzillah, N.A. and Mirghani, N.E.S. (2018). A Review of Gelatine Source Authentication Methods. *Tropical Life Sciences Research,* 29(2), 213–227. https://doi.org/10.21315/tlsr2018.29.2.15

Hassan, N., Ahmad, T. and Zain, N.M. (2018). Chemical and Chemometric Methods for Halal Authentication of Gelatine: An Overview. *Journal of Food Science*, 83(12), 2903-2911. https://doi.org/ 10.1111/1750-3841.14370

Hashim, D., Man, Y.C., Norakasha, R., Shuhaimi, M., Salmah, Y. and Syahariza, Z. (2010). Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatines. *Food Chemistry,* 118(3), 856-860. https://doi.org/10.1016/j.foodchem.2009.05.049

Hermanto, S., Sumarlin, L.O. and Fatimah, W. (2013). Differentiation of Bovine and Porcine Gelatine Based on Spectroscopic and Electrophoretic Analysis. *Journal of Food and Pharmaceutical Sciences,* 1, 68–73.

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

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 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

Lund, M.N. and Ray, C.A. (2017). Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. *Journal of Agricultural and Food Chemistry,* 65(23), 4537–4552. https://doi.org/10.1021/acs.jafc.7b00882

Malik, A., Sutantyo, M.L., Hapsari, I., Sinurat, A.V., Purwati, E.M., Jufri, M. and Suryadi, H. (2016). Simultaneous identification and verification of

gelatine type in capsule shells by electrophoresis and polymerase chain reaction. *Journal of Pharmaceutical Investigation,* 46(5), 475–485. https://doi.org/10.1007/s40005-016-0245-0

Munjanja, B. and Sanganyado, E. (2015). UV–Visible Absorption, Fluorescence, and Chemiluminescence Spectroscopy. In Nollet, L.M.L. and Toldra, F. (Ed.) Handbook of Food Analysis, 3rd ed., p. 572-583. USA: CRC Press.

Mutalib, S.A., Muin, N.M., Abdullah, A., Hassan, O., Mustapha, W.A.W. and Maskat, M.Y. (2015). Sensitivity of polymerase chain reaction (PCR)-southern hybridization and conventional PCR analysis for Halal authentication of gelatine capsules. *LWT - Food Science and Technology,* 63(1), 714–719. https://doi.org/10.1016/j.lwt.2015.03.006

Natella, F., Nardini, M., Giannetti, I., Dattilo, C. and Scaccini, C. (2002). Coffee drinking influences plasma antioxidant capacity in humans. *Journal of Agricultural and Food Chemistry*, 50, 6211–6216. https://doi.org/10.1021/jf025768c

Nur Azira, T., Ismail A. and Che Man, Y.B. (2012). Differentiation of bovine and porcine gelatinees in processed products via Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and principle component analysis (PCA) technique. *International Food Research Journal,* 19(3), 1175–1180.

Nur Azira, T., Ismail, A., Mustafa, S. and Hamid, M. (2016). Development of antipeptide enzyme-linked immunosorbent assay for determination of gelatine in confectionery products. *International Journal of Food Science and Technology*, 51(1), 54–60. https://doi.org/10.1111/ijfs.12971

Raraswati, M.A., Triyana, K. and Rohman, A. (2013). Differentiation of bovine and porcine gelatines in soft candy based on amino acid profiles and chemometrics. *Journal of Food and Pharmaceutical Science*, 2(1), 1–6.

Rohman, A., Triyana, K., Sismindari and Erwanto, Y. (2012). Differentiation of lard and other animal fats based on triacylglycerols composition and principle component analysis. *International Food Research Journal,* 19(2), 475–479.

Roussel, S., Preys, S., Chauchard, F. and Lallemand, J. (2014). Multivariate Data Analysis (Chemometrics). In O'Donnell, C., Fagan, C. and Cullen, P. (Eds.) Process Analytical Technology for the Food Industry, p. 7–59. New York: Springer. https://doi.org/10.1007/978-1-4939-0311-5_2

Rufian-Henares, J.A. and Morales, F.J. (2007). Functional properties' of melanoidins: in vitro antioxidant, antimicrobial and antihypertensive activities. *Food Research International*, 40, 995–1002. https://doi.org/10.1016/j.foodres.2007.05.002

Sudjadi, Wardani, H.S., Sepminarti, T. and Rohman, A. (2016). Analysis of porcine Gelatine DNA in a commercial capsule shell using real-time polymerase chain reaction for halal authentication. *International Journal of Food Properties,* 19(9), 2127–2134. https://doi.org/10.1080/10942912.2015.1110164

Sultana, S., Motalib Hossain, M.A., Naquiah, N.N.A. and Ali, M.A. (2018). Novel multiplex PCR-RFLP assay discriminates bovine, porcine and fish gelatine substitution in Asian pharmaceuticals capsule shells. *Food Additives and Contaminants: Part A*, 35(9), 1662-1673. https://doi.org/10.1080/19440049.2018.1500719

Tamanna, N. and Mahmood, N. (2015). Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *International Journal of Food Science,* 2015, 1-6. https://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

