SIGNIFICANCE OF THE MAILLARD REACTION FOR GELATIN HALAL AUTHENTICATION

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 ABSTRACT

The quality and authenticity of gelatin in food production become a consumer highlight nowadays. The important factors in the selection of any food products by consumers are the name, origin, and information about the ingredients used. Gelatin has been used widely as main ingredients for hydrocolloids in foods, pharmaceutical, nutraceutical and cosmetic formulations. Thus, the gelatin authentication has become an important issue among Muslims, Jews, Hindus, Vegan, and vegetarian communities. Some methods have been applied for gelatin authentication such as infrared spectroscopy, mass spectroscopy, electrophoresis, gas chromatography, high-performance liquid chromatography, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). However, these methods use high end technology, require competent skill, relatively high cost and time consuming for industrial practice. Maillard reaction or non-enzymatic browning was common in food technology. Interaction of reducing sugar with protein in Maillard reaction will produce some sensory active compounds such as color, odor, and taste depend on reactant substances and condition of reaction. Gelatin from different sources revealed different amino acids composition. Since the amino acid composition of a gelatin varies with their origin, it is possible that color and flavor compounds, when subjected to Maillard reaction will vary. The differences will be the key principle in halal authentication of gelatin.

Field of Research: Halal authentication, gelatin, maillard reaction, species specific detection

1. Introduction

Foods are very essential for our life. Improvement in technology has been affected the production of food in the world. Not only in the quantity, but also in quality and variant. Technology has been used in processing, handling, packaging, and distribution of food in the wide world. Muslims community have special needs for foods. Islam encourage all humankind to choose a good food for their consumption. In Islam, food is confined particularly to the concept of halal and haram. Halal is an Arabic word which means ‘lawful’, ‘permissible’ under Islamic law. The opposite halal is ‘haram’ which mean ‘unlawful’, ‘prohibited’ and ‘forbidden’, while mushbooh refer to doubtful of the halal status. Halal food products should not only be free from a haram constituent, but they should also be thayyib, the term given to goods or products which meet the quality standards. The term thayyib refers to a particular good or product that is clean, pure and produced based on standard processes and procedures(Ab Halim, Kashim, Salleh, Nordin, & Husni, 2015).

Food authentication including oil, fat and gelatin and detection adulteration is big issue in the food area, not only for producers, also for consumers and government. Pig and pig derivatives such as pork, lard, and gelatin, not only using as an additive but as the main material in food and pharmaceutical production. Improvement in food, pharmaceutical, and cosmetic technologies has been increasing the possibility for counterfeiting practices. For this reason, identification or analysis of pig derivatives in foods and pharmaceuticals is necessary.
Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is the structural mainstay and most common protein in the animal kingdom. Gelatin is a high molecular weight polypeptide and an important hydrocolloid, which has proved popular with the general public and finds its uses in a wide range of food products largely because of its gelling and thickening properties (Mariod & Adam, 2013). The classical food, photographic, cosmetic and pharmaceutical application of gelatin is based mainly on its gel-forming properties. However, recently, gelatin has been used as emulsifiers, foaming agents, colloid stabilizers, biodegradable film forming materials and micro-encapsulating agents, and also the source of bioactive peptides (Gomez-Guillen, Gimenez, Lopez-Caballero, & Montero, 2011).

The most abundant sources of gelatin are pig skin, bovine hide and, pork and cattle bones (Gomez-Guillen et al., 2011; Hafidz, Man, Amin, & Noorfaizan, 2011). Recently, some species have been investigated to find alternative of gelatin such as fish (Khiari, Rico, Martin-Diana, & Barry-Ryan, 2013; Monsur, Jaswir, Salleh, & Alkahtani, 2014; Silva, Bandeira, & Pinto, 2014; Yan, Li, Zhao, & Yi, 2011), chicken (Abdullah et al., 2016; Mhd Sarbon, Badii, & Howell, 2013), duck (Abedinia, Ariffin, Huda, & Mohammadi Nafchi, 2017), and goat (Mad-Ali, Benjakul, Prodpran, & Maqsood, 2016).

The primary structure and composition of gelatin resemble the parent collagen. This similarity has been substantiated for several tissues and species. Slight differences are due to the source of raw material in combination with the pre-treatment and extraction procedures used. There are two processes by which collagen is processed to gelatin. The acid process is mainly used for pig skin and fish skin and sometimes bones raw materials. In this process, collagen is acidified to about pH 4 and then heated, denatured, defatted, filtered, concentrated, then drying by passing dry air over the gel. The obtained product is ground and blended with customer requirements and packed. The alkali process is used on bovine hide and collagen sources; in this process, collagen is submitted to a caustic soda or lengthy liming process prior to extraction. After the alkali processing, the collagen is washed and treated with acid to the desired extraction pH. The collagen is then denatured and converted to gelatin by heating, then vacuum evaporated, filtered, gelated, dried, grind and blended (Mariod & Adam, 2013).

Industrial gelatins are mixtures of different compounds: a-chains (one polymer chain), b-chains (two a-chains covalently crosslinked), and g-chains (three covalently crosslinked a-chains) (Karim & Bhat, 2009). The amino acid composition and sequence in gelatin are different from one source to another but always consists of large amounts of glycine, proline, and hydroxyproline. It is repeated with typical sequence, Gly-X-Y where glycine is the most abundant amino acid in gelatin; X and Y are mostly proline and hydroxyproline, respectively. According to Hafidz & Yaakob (2011), 25% of dry gelatin contains proline and hydroxyproline that stabilize its structure.

2. Gelatin Authentication

The analysis on halal authentication of porcine gelatin from other sources of gelatin has been studied over the years. Detection and characterization methods rely on protein analysis or physicochemical properties such as chemical precipitation, Fourier transform infrared spectroscopy (Cebi, Durak, Toker, Sagdic, & Arici, 2016; Hermanto, Sumarlin, & Fatimah, 2013), electrophoretic (Hermanto et al., 2013), high-performance liquid chromatography, mass-spectrometer detection (Grundy et al., 2016; Zhang et al., 2009), and enzyme-linked immunosorbent assay (Tukiran, Ismail, Mustafa, & Hamid, 2016a), polymerase chain reaction (Ali, Razzak, Bee, & Hamid, 2014; Shabani, Mehdizadeh, Mousavi, et al., 2015) have been applied to differentiate bovine gelatin from porcine gelatin.

In precise, eight studies have been published from 2003 to 2010 in various application of analytical methods regarding spectroscopic, chemical precipitation, liquid chromatography and immunochemical techniques (Hafidz, Ismail, & Man, 2012). To the best of found knowledge, the expansion of the authentication methods of gelatin from 2010 and onwards result in another eleven studies (Table 1). The variation of authentication of methods is according to the principles, advantages, and limitation of each method. Most of the methods use Principal Component Analysis (PCA) to
facilitate the discriminant analysis of different sources of gelatin. Besides, nearly all methods approached two types of samples which are the raw material of gelatin and food product that contains gelatin for validation of the methods in industry later for both samples; the raw ingredient and end-product.

Table 1: The method for halal authentication of gelatin from 2012 to 2016.

<table>
<thead>
<tr>
<th>Method</th>
<th>Technique/Instrument</th>
<th>Biomarker/Principle</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-based</td>
<td>PCR</td>
<td>DNA sequence</td>
<td>(Demirhan, Ulca, &amp; Senyuva, 2012; Mutalib et al., 2015; Shabani, Mehdizadeh, Mohammad, &amp; Ansari, 2015)</td>
</tr>
<tr>
<td>Protein-based</td>
<td>ELISA</td>
<td>Anti-peptide polyclonal antibody</td>
<td>(Tukiran, Ismail, Mustafa, &amp; Hamid, 2016b)</td>
</tr>
<tr>
<td></td>
<td>SDS-PAGE</td>
<td>Polypeptide</td>
<td>(Azira &amp; Man, 2012; Hermanto et al., 2013; Nur Azira, Che Man, Raja Mohd Hafidz, Aina, &amp; Amin, 2014)</td>
</tr>
<tr>
<td>Analytical</td>
<td>HPLC</td>
<td>Peptide/Chromatography Spectroscopy</td>
<td>(Azilawati, Hashim, Jamilah, &amp; Amin, 2015; Yilmaz et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>FTIR</td>
<td>Spectroscopy</td>
<td>(Cebi et al., 2016; Hermanto et al., 2013)</td>
</tr>
<tr>
<td></td>
<td>UV-Vis</td>
<td>Spectroscopy</td>
<td>(Hermanto et al., 2013)</td>
</tr>
<tr>
<td>Chemical reaction</td>
<td>Maillard reaction</td>
<td>Chemical compound</td>
<td>(Tan, Alkarkhi, &amp; Mat, 2012)</td>
</tr>
</tbody>
</table>

2.1. DNA-based method

The biomarker of deoxyribonucleic (DNA) in porcine and bovine gelatin can be determined by using Polymerase Chain Reaction (PCR) that focus on a segment of DNA and copy it billion overtimes. For real-time PCR, the minimum detection level of adulteration of porcine in marshmallows and gum reached 1.0% w/w (Demirhan et al., 2012). The positive result of porcine DNA acquired from two over fourteen samples and one over twenty-nine samples from Germany and Turkey respectively, with the absence of an indication of porcine gelatin on the product label. Besides, the lowest detection level of porcine and bovine gelatin had been studied is 0.1% w/w by species-specific PCR (Shabani, Mehdizadeh, Mohammad, et al., 2015). The sensitivity of the method was tested on binary gelatin mixtures between both sources containing 0.1%, 1%, 10%, and 100% (w/w) with the amplification of 212 bp for porcine gelatin and 217 bp for bovine gelatin.

In addition, some researcher uses PCR with southern hybridization on-chip that can detect 6 porcine DNA over 20 samples of gelatin capsule which not available by conventional PCR analysis (Mutalib et al., 2015). This is due to the sensitivity of primers of porcine DNA are 0.25 ng (cyt b) and 0.001 ng (Olipro™ Chip) make it more effective compared to conventional PCR. A DNA-based method is the preferable one for it highest sensitivity and accuracy. However, this method required extraction and purification of DNA from gelatin and few of porcine specific primers on a multicopy target on mitochondria DNA (mtDNA) of cytochrome b (cyt b). Severe processing steps of gelatin production that cause the degradation most of the DNA should take into consideration.

2.2. Protein-based method

Next, the specializations in protein analysis are Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Enzyme-Linked Immunosorbent Assay (ELISA). In SDS-PAGE with Principal Component Analysis (PCA), the study focuses on gelatin polypeptides from porcine and
bovine with molecular weight ranged from 53 to 220 kDa (Tukiran & Man, 2012). About 11 prominent polypeptides found in porcine gelatin which differ than 2 prominent polypeptides of bovine gelatin. This method requires efficient extraction of polypeptides from gelatin by using a solvent such as cold acetone and deionized water. However, this combination method could only stipulate rough information for gelatin species differentiation in processed foods. The partial degradation and interaction of gelatin with other proteins in the processed foods may lead to poor electrophoretic profile.

In addition, Hermanto, Sumarlin& Fatimah (2013) utilize SDS-PAGE with pepsin hydrolysis for gelatin authentication by identification of molecular weight of gelatin fragment. Pepsin can cleavage up to 20% of amide bonds of gelatin at the N-terminal of aromatic amino acids such as phenylalanine, tryptophan, and tyrosine. Although the peptide pattern of two sources of gelatin can be studied, the real differentiation between them only shown by two peptide fragments of porcine gelatin with molecular sizes below 36.2 kDa and 28.6 kDa after 2 hours incubation.

Second, the competitive indirect enzyme-linked immunosorbet assay (ELISA) has complimentary with the SDS-PAGE technique. The ELISA technique detects and quantifies peptides, proteins, antibodies and hormones in a plate-based assay. Being specific, an antigen should be immobilized on a solid plate surface before an antibody-enzyme linkage should be complexed to assess enzyme activity with the substrate. Nevertheless, high similarity of amino acid sequences of collagen from different species and low antigenicity of gelatin would make their immunochemical differentiation troublesome (Venien & Levieux, 2005).

Thus, appropriate antigen at a molecular weight of 125-kDa is chosen from the result of prominent bands of porcine gelatin by SDS-PAGE (Tukiran et al., 2016b). After that, the polyclonal antibody was raised against a porcine species-specific amino acid sequence of collagen α2 (I) chain. The ELISA technique has an advantage for gelatin in confectionary products since the collagen α2 (I) chain protein involved has resistance against heat treatment. The value of the concentration of an inhibitor where the binding is reduced by half (IC50) is 0.39 μg mL−1 and the limit of detection (IC10) is 0.05 μg mL−1. Through this method, mammal and fish gelatin can be differentiated as fish and chicken gelatin shown low cross-reactivity.

2.3. Analytical Method

High-Performance Liquid Chromatography (HPLC) can be utilized for detection of amino acids (Azilawati et al., 2015) and peptides (Yilmaz et al., 2013). Chromatography technique can separate a mixture of bombarded molecules in mobile phase according to their molecular weight into the stationary phase in the column. It is important for this instrument to have the internal library for the detection. Thus, the amino acid analysis and relationships from bovine, porcine and fish gelatin can be determined by using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate as derivatization reagent and Principal Component Analysis (PCA) respectively. Loadings plot and scores plot of PCA model shown that threonine, serine, and methionine are correlated to fish gelatin while polar and non-polar amino acids are respectively correlated to porcine and bovine gelatin.

Another novel method using high-end instrument of HPLC is Ultra-Performance Liquid Chromatography and Electrospray Ionization Quadrupole Time-Of-Light Mass Spectrometry (NanoUPLC-ESI-q-TOF-MSE). The peptides work as biomarkers are suitable for detection of porcine and bovine gelatin in yogurt, cheese, and ice cream products. The separation and analyzation enabled accurate mass acquisition on the peptide due to data independent acquisition mode along with substitution of collision energy (low and high) to render the precursor ion and product ion information. However, this method requires tryptic gelatin peptides extraction from both gelatins.

Other than that, gelatin profile from an animal can be distinguished by using Fourier Transform Infrared (FT-IR) and Ultraviolet-Visible (UV-Vis) Spectroscopy. Interestingly, both method use spectroscopy
principle where the amount of light absorbed due to molecule vibrations over a range of frequencies of the incident light will be measured by the different type of electromagnetic wave. The spectra of fish, porcine and bovine gelatin solution obtained from Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) can be clearly classified and differentiate with Hierarchical cluster analysis and Principal Component Analysis (PCA) (Cebi et al., 2016). The chemometric method is shown effectiveness at Amide-I (1700–1600 cm\(^{-1}\)) and Amide-II (1565–1520 cm\(^{-1}\)) spectral bands. Besides, the pure bovine gelatin is discriminable from the mixture of bovine and porcine gelatins representing the adulteration in food, pharmaceuticals and cosmetics products.

Beforehand, this method also studied by Hermanto, Sumarlin & Fatimah (2013) for pepsin-hydrolyzed gelatin from bovine and porcine. The spectra between two sources of gelatin fragment are slightly different mainly in the region of 2800-3000 cm\(^{-1}\) (aliphatic C-H stretching), 1543 cm\(^{-1}\) (C-N-H bending), 1450-1300 cm\(^{-1}\) (C-H bending) due to the different amino acid composition of the two sources of gelatin. Although FTIR method is rapid and simple for gelatin authentication it requires a lot of data and high purification of a sample. Thus, gelatin fragments must be dried up in a freeze drier to remove impurities such as solvent and salts. UV-Vis Spectroscopy also uses for the same samples to differentiate the chromophore groups profiles from the two different source of gelatin fragments (Hermanto, Sumarlin & Fatimah, 2013). In the species comparison, the absorbance profiles are slightly different in the wavelength range 210-240 nm due to different amino acids composition. Obviously, the absorbance of gelatin solution before hydrolysis (229 nm) and after hydrolysis (240 nm) for both gelatins is different. The gelatin has the different proportion of C=O amide and two-dimensional conformation after hydrolyzed into a peptide.

2.4. Chemical reaction method

The different value of pH, color (Commission Internationale de l’Eclairage, CIE L* and b*) and absorbance (A\(_{420}\)) of Maillard reaction products is studied for gelatin identification, differentiation and quality control (Tan et al., 2012). This reaction is indicated by the formation of a brown solution after heating gelatin with sugar. Gelatin identification of two types of gelatin can be performed by cluster analysis. Meanwhile, gelatin differentiation can be determined by confidence interval (95% confidence) between the values of absorbance and color of Maillard reaction product. The application of Statistical Process Control (SPC) can evaluate the gelatin for the assessment of quality control. The potential use of the method as a quality control procedure and good reproducibility can be evaluated by using SPC. Further study on the Maillard reaction product can utilize this method for gelatin authentication from different species.

3. Maillard Reaction

3.1. Definition and Application

The Maillard reaction is a complex series of chemical reactions that occur naturally during food processing and storage at the higher temperature (Cerny, 2008; Q. Liu, Niu, Zhao, Han, & Kong, 2016). In Maillard reaction, an interaction between sugar and amino acids with the presence of heat will produce some chemical substances, namely Maillard Reaction Products (MRPs). Products of Maillard reaction can be some high molecular mass compounds and the lower molecular mass compounds (Shen, Tseng, & Wu, 1999). Maillard reaction products have effect on nutritive value (Gu, Abbas, & Zhang, 2009; Małgorzata, Konrad, & Zielin, 2016; Thomas & Forbes, 2010; Yang et al., 2015), toxicological implication (mutagenic and carcinogenic) (Jaeger, Janositz, & Knorr, 2010) and antioxidative components (Gu et al., 2009; Q. Liu et al., 2016; Małgorzata et al., 2016).

Currently, some research has been developed to investigate the effect of Maillard reaction on foods. The study on color formation on food products were reported for peptide (Kim & Lee, 2009), cornflakes (Farroni & Buera, 2012), cane brown sugar (Asikin et al., 2014), buckwheat (Małgorzata et
al., 2016), and soybean hydrolysate (M. Yu et al., 2018b). Study on flavour compounds of Maillard reaction products were reported for cake (Rega, Guerard, Delarue, Maire, & Giampaoli, 2009), bread (Prost, Poinot, Rannou, Arvisenet, & Université, 2012), ghee (milkfat) (Andrewes, 2012), soy sauce (Feng et al., 2015; Inoue et al., 2016), cocoa (Taş & Gökmen, 2016), cook cured pork ham (Benet et al., 2016) and also peptide (J. Liu, Liu, He, Song, & Chen, 2015; Ogasawara, Katsumata, & Egi, 2006; R. Wang, Yang, & Song, 2012).

Hodge subdivides the Maillard reaction into 3 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 second stage is the intermediate stage which includes sugar degradation, sugar fragmentation and amino acid degradation (Strecker degradation) and the last stage is the final stage which includes aldol condensation, aldehyde-amine condensation, and formation of heterocyclic nitrogen compounds (Thomas & Forbes, 2010).

The Maillard reaction is influenced by many factors. The factors including types of amino acids and sugars, temperature, time, pH and water activity (Kim & Lee, 2009; Lee, Chung, & Kim, 2012). The type of amine and carbonyl compounds influence the rate of reaction as well as the products formed. Kim & Lee (2009) reported that the Maillard reaction is greatly affected by the peptide chain length. Thus, the Maillard reaction rate might be related to the degree of hydrolysis of the peptide bond and the stability of the peptide bond as the heating time increased. The concentration and ratio of reactants also significantly changed the Maillard reaction trend (Bell, 1997; Chen & Kitts, 2008; Guan, Wang, Yu, Yu, & Zhao, 2012; Martins & Van Boekel, 2005). In addition, the pH value significantly influences the Maillard reaction rate and the types of products formed. High pH in Maillard reaction suggested to the main pathway to flavor formation (Martins & Van Boekel, 2005; A. Yu & Zhang, 2010b).

3.2. The Significance of Maillard Reaction for Gelatin Authentication

The study on sensory active compounds of Maillard reaction product was reported by many researchers. In the food processing, the Maillard reaction will produce various sensory-active compound as well as color, odor, and taste (Cerny, 2008). Basically, the study on Maillard reaction system developed based on sugar-protein/peptide and sugar-amino acid model. Several studies have shown that the amino acids compositions of gelatin vary according to the species (R. M. R. . Hafidz et al., 2011; Karim & Bhat, 2009). It is possible that the amino acids in gelatin responded differently during the Maillard reaction process. Based on this presumption, some models of Maillard reaction can be developed for gelatin authentication based on the abundance of amino acids.

Tabel 2. Models Maillard reaction for gelatin authentication based on the abundance of amino acids

<table>
<thead>
<tr>
<th>Sensory characteristic</th>
<th>Model Maillard reaction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown color</td>
<td>Asparagine-glucose</td>
<td>Yuan, Hu, Jie, &amp; Fang, 2008</td>
</tr>
<tr>
<td></td>
<td>Proline-glucose</td>
<td>Guan et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Glycine-fructose</td>
<td>Guillermo &amp; Reyes, 1982;</td>
</tr>
<tr>
<td></td>
<td>Glycine-ribose</td>
<td>Chen &amp; Kitts, 2008</td>
</tr>
<tr>
<td>Flavor compounds</td>
<td>Proline-glucose</td>
<td>Tressl et al, 1985</td>
</tr>
<tr>
<td></td>
<td>Proline-rhamnose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proline-arabinose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proline-erythrose</td>
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</tr>
</tbody>
</table>
3.2.1. Color

Color formation is the main characteristic of the Maillard reaction. The brown color from Maillard reaction is known as melanoidins. Melanoidins are the polymer with high molecular weight usually contain 3-4% nitrogen (Forbes, 2010). They can absorb light at wavelengths as high as 420 nm and are predominantly responsible for the characteristic brown color of foods such as coffee, cocoa, bread, malt, and honey.

The complex array of melanoidins produced in the Maillard reaction is strongly dependent on the type of food, as well as the technological conditions of the reaction such as treating temperature and time, pH, solvent, and the composition of amino acids and reducing sugars (Jaeger et al., 2010; Van Boekel, 2006; H. Y. Wang, Qian, & Yao, 2011). According to Kwak & Lim (2004), the color intensity of MRPs from basic amino acids was reportedly greater than that of acidic amino acids, while nonpolar amino acids were of intermediate color intensity. In addition, browning was accelerated by the presence of metal ions (Fe$^{2+}$ and Cu$^{2+}$) but inhibited by Na$^+$. Study on brown color of gelatin for species-specific differentiation purpose reported by Tan, Alkarkhi, & Easa (2012) where the bovine and porcine gelatins were successfully differentiated with UV-spectroscopy reading after ribose-induced Maillard reaction. Hamizah et al. (2017) reported that the presence of Cu$^{2+}$ during the non-enzymatic browning of gelatin samples causes an increase in the rate of browning index more than 100%. The effect of Cu$^{2+}$ on changing of the browning index of the three gelatin samples (porcine, bovine and fish gelatins) was similar such that changing of the browning index increased drastically until the 12 h of heating for bovine and porcine gelatin, while that of fish gelatin slightly decreased to about 100%.

Some models sugar-amino acid can be developed for gelatin authentication (See Table 2). Most of the study focuses on the glycine-glucose model, whereas the glycine is the simple amino acid. This model more adaptive to gelatin authentication for some reasons: the abundance in gelatin (Hafidz et al., 2011), solubility in water and ethanol (Shen et al., 1999), glucose more reactive than fructose in the whole reaction with glycine (Guillermo & Reyes, 1982). In addition, the standard of melanoidin was developed by this model (Thomas & Forbes, 2010). Based on the studies above, the system for melanoidin formation in glycine-glucose models adjusted to acidic condition (pH 3.5-6.7) and low temperature ($\leq$ 95°C).

Another study by Chen & Kitts (2008) used equimolar amounts of glycine-ribose, lysine-ribose, glycine-glucose, and lysine-glucose were heated at 121°C for 60 minutes under pH 7 shown that the product contains fluorescence and antioxidant substances. Pentozone, 3-deoxypentosone (3-DP), Glyoxal (GO), and methylglyoxal (MGO) were identified in glycine-ribose and lysine-ribose models, while glucosone,3-deoxyglucosone (3-DG), GO, and MGO were identified in Glc–Gly, and Glc–Lys model systems. Maillard reaction product from glycine-ribose model has greater antioxidant capacity than glycine-glucose.

3.2.2. Flavor

Flavor compound is another of the characteristics of Maillard reaction products. It is a complex sensation, made up principally of smell and taste. In general, odor thresholds are much lower than taste threshold and so flavor tends to be dominated by odor components. The profile of volatile compounds in a sample is unique and can be easily destroyed by the simple process (Marsili, Drake, & Miracle, 2007). This principle can be developing for efficient analytical techniques for the monitoring of volatile compounds or flavor components in food products. Most of the research on the formation of Maillard-based flavor compounds is on sugar–protein or sugar–peptide mixtures, and hardly on mixtures of sugar and free amino acids. However, no study reported on flavor formation of Maillard reaction for gelatin model.
Flavour compound formation in the Maillard reaction depends on the type of sugars and amino acids involved (Van Boeke, 2006), reaction temperature (Jaeger et al., 2010; Van Boeke, 2006; A. Yu, Tan, & Wang, 2012; A. Yu & Zhang, 2010a), time (Gong et al., 2017; J. Liu et al., 2015; A. Yu et al., 2012), pH (A. Yu & Zhang, 2010b) and water content (Van Boeke, 2006). Thermal treatment is one of the most important factors affecting the reaction rate and flavoring characteristics of the Maillard reaction (Jaeger, 2010). At relatively low temperatures (80-100 °C), the odor intensity of basic meaty and nutty/roast was increased slowly. However, at 120 °C, pyrazines as flavor compounds increased rapidly along with the increase of heating time (J. Liu et al., 2015; A. Yu et al., 2012). According to Thomas & Forbes (2010), the formation of volatile compounds by the Maillard reaction can be classified into 3 groups: simple sugar dehydration/fragmentation products, simple amino acid degradation products and volatiles produced by further interactions.

The volatiles formed from the simple sugars reaction with proline was analyzed in 1985. Tressl and coworkers used the simple sugars (erythrose, arabinose, glucose, and rhamnose) which was heated separately with equimolar amounts of proline. From this reaction, pyrrolidines, piperidines, pyrrolizines, and azepines were identified as proline specific Maillard reaction product.

Peptides play an important role in the taste of foods. Strong umami, mouthfulness, continuity, meaty attributes have been demonstrated in cysteine-xylose-soybean peptide model system, as mentioned by Huang et al., 2011. The similar finding report by Song et al., 2016, for beef bone protein, and Inoue et al., 2016 for soy miso. Ogasawara et al., (2006) reported that the Maillard peptides with a molecular weight of 1,000–5,000 Da generated during the aging of soybean paste affected not only the basic taste qualities but also enhanced mouthfulness and continuity.

4. Conclusion

Formation of brown color and flavor components in Maillard reaction can be developed as the alternative method in protein authentication, especially for gelatin. Since the product of Maillard reaction affected by the type of amino acid and sugar, many models of Maillard reaction can be investigated. The difference in amino acid composition in gelatin from the different source will produce different products when subjected to Maillard reaction. The further analysis can be developed based on the browning index, odor profile, taste and fluorescence substances with the relevant instrument.
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