

## Study on Volatile Compounds of Gelatine and The Maillard Reaction Products from Different Species Using SPME-GCMS

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### Abstract

Food fraud and adulteration are the global issues, currently. One important issue is about gelatine since it comes from many sources of animals. Therefore, analytical method for gelatine must be developed and used for the authentication. This study was conducted to investigate the Volatile Compounds (VCs) of gelatine and the Maillard Reaction Products (MRPs) which are responsible to their aroma. Three gelatine standards from bovine, fish and porcine bought from Sigma Aldrich were used. A high reactivity reducing sugar namely xylose was used in the Maillard Reaction (MR). A Solid Phase Micro Extraction-Gas Chromatography-Mass Spectroscopy (SPME-GC-MS) used to evaluate the VCs in the samples. The VCs were identified by comparing the mass spectra of the compounds with database of NIST library. Moreover, retention time using the n-Alkane index were compared with literature data. There were 67 VCs have been identified. Among them, furfural, acetic acid, nonanone, dimethyl disulphide and decanone were considered as the important volatiles in gelatine due to its abundance. In the MRPs, furfural, 1-(2-furanylmethyl)-1H-pyrrole, 1-(2-furanyl)-ethanone, acetic acid and 2,2'-bifuran were predominant. Additionally, heptanol, octanal, nonanal, nonanone, dimethyl disulphide and dimethyl trisulphide could be considered as the important compounds due to its low odour threshold value. The compounds had a direct influence on the overall aroma of samples and could be potential to use in gelatine differentiation.

### Keywords

Gelatine, Maillard Reaction, Aroma, Flavour Analysis, SPME-GCMS

Received: 27 Oktober 2021, Accepted: 4 February 2022

<https://doi.org/10.26554/sti.2022.7.2.132-139>

## 1. INTRODUCTION

Protein has a contribution to the nutritional properties of foods through the provision of amino acids that are essential to human growth (Li-Chan and Lacroix, 2018). As a pure protein, gelatine has a functional role in living organisms. In this age, gelatine has been widely applied in foods, pharmaceuticals, nutraceuticals and cosmetics products. The classical food, photographic, cosmetic and pharmaceutical application of gelatine is based mainly on its gel-forming and thickening properties (Gómez-Guillén et al., 2011; Mariod and Fadul, 2013). It has been used as an emulsifier, colloid stabilizers, foaming agents, micro-encapsulating agent, biodegradable film-forming materials and as source of bioactive peptides (Chung, 2020; Gómez-Guillén et al., 2011).

Gelatine is still being an issue among the consumers due

to cultural, healthy reason and religious due to its source of origins. It was reported by Transparency Market Research (TMR) that by 2018, the global gelatine market is estimated to reach 450.700 kilotons, up from 348.900 kilotons of production capacity in 2011. According to another source, the world gelatine market was valued at 620.600 kilotons in 2019 and is projected to expand at a volume-based compound annual growth rate of 5.900% from 2020 through 2027. However, halal gelatine in the global market is less than 2%. The most abundant source of gelatine derived from porcine skin (80%), followed by bovine hide (15%), and porcine bone, cuttle bone, and fish (5%) (Tongdeesoontorn and Rawdkuen, 2019).

The unclear information and labelling of gelatin in the market increase consumers concern over its halal authenticity. In concern with food safety, analytical laboratory approach needs to be done in order to know chemically additive substances that

may contain in the food product. Currently, several methods have been developed for halal authentication purpose in gelatine. Spectroscopy, chromatography, DNA and protein-based methods such as ELISA and PCR are mostly used. It is well known that the methods discussed are successful, accurate and precise, but the advantages of these methods are that the studies require expertise and can be time consuming. In addition, toxic chemicals are used for destruction and extract the samples (Cebi et al., 2019).

Some reports find that UV-spectroscopy techniques have been successfully applied in the differentiation of bovine and porcine gelatine (Tan et al., 2012; Nurrulhidayah, 2019). As an authentication method, the Maillard Reaction (MR) was introduced, which developed the browning effect in gelatine. Bovine and porcine gelatine were distinguished by the differences in the MR browning intensities. However, there were limited reports about other aspects of the gelatine-MRPs, such as aroma and taste. Therefore, the purpose of this study was to investigate the VCs of gelatine-MRPs which may be useful as aroma indicators for determining gelatine sources.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

Three different types of gelatine, namely bovine skin gelatine (G9391: type B, powder, bio reagent), porcine skin gelatine (G1890: type A, powder, bio reagent) and fish skin gelatine (G7041: bio reagent, solid) were used in this study. D-Xylose (W360600: crystalline) was used as reducing sugar in the MR. Gelatine and xylose were purchased from Sigma Aldrich Germany. Natrium hydroxide was purchased from Merck was used for set the pH of mixtures.

### 2.2 Methods

#### 2.2.1 Sample Preparation

The standard gelatine from three different origin sources (bovine, fish and porcine) was used during the experiment. Two kinds of treatments, wet and dry methods were performed. For the wet method, gelatine solution was used, while in dry method, gelatine powder was used. The method for preparation is previously described in the literature (Ismarti et al., 2020). About 5 mL of gelatine solution from three different sources (5% w/v, pH 10.9) were mixed with 0.750 g of xylose in screw seal tubes. After homogenizing the tubes and heating them at 140°C for 38 minutes in an oil bath, the samples were immediately cooled under tap water to stop the reaction. Furthermore, samples were diluted until 100 mL using ultrapure water. All samples were prepared for 10 replications. For control, the gelatine solution without xylose was prepared with the same treatment. Additionally, for the dry gelatine and MRP samples, about 2 g of samples were prepared from gelatine and xylose with a ratio of 1:2. The homogenated mixtures prepared in the same way as described for the solution samples.

#### 2.2.2 Characterization of The VCs by Using HS-SPME-GCMS

The VCs in gelatine and the MRP were determined using a HS-SPME (DVB/CAR/PDMS) (Supelco) coupled with GCMS (Agilent 7890A, Agilent Technologies, Palo Alto, CA) using DB wax capillary column (J&W122-7032:4534.42388) following the method as reported by Karnjanapratum and Benjakul (2017) with slight modification. For headspace analysis samples were heated at 60°C for 30 minutes and the VCs were extracted using DB wax capillary column. The samples were desorbed for 10 minutes on an Agilent (Agilent 7890A, Agilent Technologies, Palo Alto, CA). The injector was held constant at 260°C The GC oven temperature was held for 2 minutes at 70°C then ramped to 240°C for 10°C /minute. A 30 m, 250  $\mu$ m, 0.250  $\mu$ m column DB wax (J&W122-7032:4534.42388) capillary column with the film was used with helium as the carrier gas under a constant flow of 1 mL/minute. Total GC cycle time consists of 26 minutes run and a 5 minutes cool-down period. The mass spectrometer was operated in scan mode from m/z 29 to 550.

#### 2.2.3 Data Analysis

A comparison of the mass spectra of the VCs in the samples with the National Institute of Standard and Technology database (NIST library, Gaithersburg, MD, USA) and the retention time with literature data using n-Alkane index enabled the identification of VCs. Molecular identification was performed based on a similarity over 85% between the molecules of each peak and the given molecules in the NIST library. C<sub>8</sub>-C<sub>230</sub> alkane series from Fluka was used for Linear Retention Indices (LRI) calculation. The LRI were calculated using Equation (1) (Liu et al., 2015).

$$\text{LRI} = 100N + 100n \frac{tR_a - tR_N}{tR_{N+n} - tR_N} \quad (1)$$

Where N denotes the carbon number of the lower alkane and n denotes the carbon number difference between the two n-alkanes that bracket the component;  $tR_a$ ,  $tR_N$  and  $tR_{N+n}$  represent the retention times of the unknown component, the lower alkane and the upper alkane, respectively. The comparative analysis of gelatine and the MRP was conducted for the VCs detected in the samples.

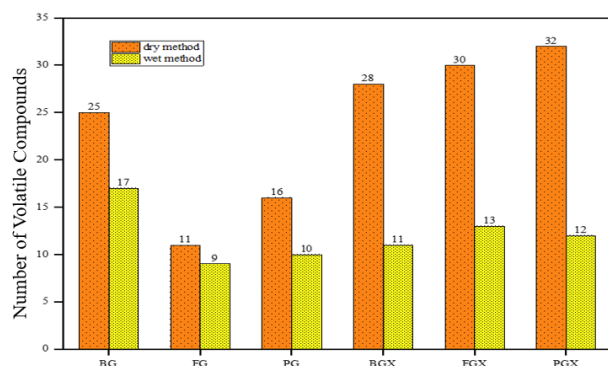
## 3. RESULTS AND DISCUSSION

The VCs of bovine, porcine, fish gelatine and the MRP were evaluated by SPME GC-MS. The differences generated among species of samples were based on the interaction between VCs and stationary phase, as indicated by the certain retention times and ion mass, referred to as NIST 4.21 library. There are three types of VCs that arise following heat treatment (Nursten, 2005): (1) amino acid degradation products (e.g., aldehydes), (2) sugar fragmentation products (e.g., furans, pyrones, cyclopentenes, carbonyls and acids), and (3) volatiles produced by further interactions (e.g., pyrroles, pyridines, imidazoles,

**Table 1.** Relative Content of Group of Volatile Compounds Detected in Gelatine and The MRPs

Sample	Relative content of volatiles based on group of compounds (%)								
	Alcohols	Acids	Esters	Aldehydes	Ketones-lactones	Furans	Pyrroles	Others	
Gelatine	BG dry	0.025	0.080	-	4.980	2.785	0.035	0.270	1.925
	FG dry	0.010	5.785	-	6.080	0.215	0.030	0.085	2.555
	PG dry	0.015	0.635	-	7.305	0.325	0.025	0.075	0.115
	BG wet	0.810	-	0.025	3.365	5.250	-	0.385	0.180
	FG wet	0.090	0.685	0.020	2.270	0.080	0.085	-	0.075
	PG wet	-	0.355	0.010	3.800	0.480	0.030	0.385	0.085
MRPs	BGX dry	2.025	1.815	0.420	31.495	4.595	7.035	9.420	-
	FGX dry	1.850	2.605	0.395	40.040	3.415	6.770	1.100	1.070
	PGX dry	2.425	1.100	0.255	45.380	3.190	6.650	7.535	-
	BGX wet	-	1.055	0.030	54.240	1.705	1.955	-	-
	FGX wet	-	3.290	0.070	48.905	1.560	1.590	-	0.005
	PGX wet	-	0.145	0.020	58.270	1.880	0.205	-	0.045

pyrazines, oxazoles and thiazoles). Total number of volatile compounds detected in gelatine and MRPs are presented in Figure 1.



**Figure 1.** Total Number of Volatile Compounds Detected in Bovine, Fish and Porcine Gelatine and The MRPs. BG: Bovine Gelatine; FG: Fish Gelatine; PG: Porcine Gelatine; BGX: Bovine-MRPs; FGX: Fish-MRPs; PGX: Porcine-MRPs

A total number of 67 VCs from different gelatine and the MRPs were detected. Generally, most of VCs were produced by samples under dry method, especially from the MRPs. The highest number of VCs was resulted from the dry-MRPs, which are porcine, bovine and fish with the 32, 30 and 28 compounds, respectively.

The relative contents for each group of VCs are presented in Table 1. The detected VCs from different groups were alcohols, organic acid, esters, aldehydes, ketones-lactones, furans, pyrroles and others (sulphur compounds, amide, pyrazine, oxazole). There was a considerable difference in the samples before and after the MR, which could be mainly attributed to the generation of novel volatiles and similar volatile substances in different concentrations (Lan et al., 2017)).

The first three main VCs in gelatine are aldehydes, acids,

and ketones-lactones groups. Of these substances, aldehyde was the most predominant, with the relative content at the range of 2.270 to 7.305%. The highest level of aldehydes for both methods was generated by porcine gelatine. A total percentage of acids was at the range of 0.080 to 5.785%, with the highest content in fish. However, this compound was not detected in porcine under wet method. Furthermore, the content of ketones-lactones was at the range of 0.080 to 5.250%, with the highest content in bovine. Other compounds, such as furans, pyrroles, alcohols, esters and others, were obtained at a relative lower level, but esters were undetected in gelatine samples under dry method.

Generally, the end products of the MR include colour, volatile aroma compounds and non-volatile products affected by temperature, water content, pH and heating time (Ikan, 1996). In addition, the presence of water affected the propagation of the MR in gelatine specially on competitive and consecutive reactions. According to Bertrand et al. (2015), such reaction did not occur in very weak water activities due to the reducing solubility of reactants, while at the high-water activities, the first dehydration stage is largely inhibited.

The predominant volatile compounds in the MRPs were aldehydes, pyrroles and furans groups. Aldehydes was detected as the abundant volatile compounds in the MRPs with the range of 31.495 to 58.270%. The amount was known to be highest in the porcine under both methods. Pyrroles were detected at the range of 1.100 to 9.420% in dry MRPs, and undetected in the samples under wet method. In addition, furan was found in a relative content at a range of 0.025 to 7.050% in all samples. The highest level of furans for both methods was found in the bovine, followed by fish and porcine. Other compounds, including alcohols, acids, esters, ketones-lactones and other compound groups were detected in smaller amount. However, no alcohols were detected in samples under wet method.

When subjected to the MR, the level of alcohols increased slightly by 81 fold for BG, 185 fold for FG and 161 fold for

**Table 2.** Relative Content of Group of Volatile Compounds Detected in Gelatine and The MRPs

Volatile compounds	RT	LRI	Gelatine						MRPs					
			BG dry	BG wet	FG dry	FG wet	PG dry	PG wet	BGX dry	BGX wet	FGX dry	FGX wet	PGX dry	PGX wet
1-Heptanol <sup>a</sup>	9.247	1562	-	0.495	-	-	-	-	-	-	-	-	-	-
1-Hexanol, 2-ethyl- <sup>a</sup>	9.654	1591	-	0.315	-	0.090	-	-	-	-	-	-	-	-
1,4-Benzenediol, 2-methyl- <sup>b</sup>	10.501	1655	-	-	-	-	-	-	-	0.035	-	0.030	-	0.045
2-Furanmethanol <sup>b</sup>	11.849	1763	-	-	-	-	-	-	-	1.990	-	0.925	-	1.075
3-Pyridinol <sup>b</sup>	13.198	1939	-	-	-	-	-	-	-	-	-	0.895	-	1.020
Benzyl Alcohol <sup>a,b</sup>	14.336	2083	0.015	-	-	-	-	-	-	-	-	-	-	0.285
Phenol, 4-methyl- <sup>a</sup>	16.415	2286	-	-	-	-	0.010	-	-	-	-	-	-	-
Phenol, 2,4-bis(1,1-dimethylethyl)- <sup>a</sup>	18.522	2513	0.010	-	0.010	-	0.005	-	-	-	0	-	-	-
<b>Number of alcohols</b>			<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>4</b>	<b>0</b>
Acetic acid <sup>a,b</sup>	9.167	1556	0.080	-	5.785	0.685	0.300	0.355	1.730	0.965	2.605	3.290	0.980	0.125
Propanoic acid <sup>a,b</sup>	10.363	1645	-	-	-	-	0.090	-	-	0.090	-	-	-	0.020
Butanoic acid <sup>a</sup>	11.459	1731	-	-	-	-	0.245	-	-	-	-	-	-	-
Hexanoic acid <sup>b</sup>	14.017	2054	-	-	-	-	-	-	0.020	-	-	-	0.010	-
Octanoic acid <sup>b</sup>	16.210	2266	-	-	-	-	-	-	0.065	-	-	-	0.025	-
Dodecanoic acid <sup>b</sup>	20.072	2693	-	-	-	-	-	-	-	-	-	-	0.005	-
Tetradecanoic acid <sup>b</sup>	21.862	2907	-	-	-	-	-	-	-	-	-	-	0.080	-
n-Hexadecanoic acid <sup>b</sup>	24.219	3118	-	-	-	-	-	-	-	-	-	-	0.050	-
<b>Number of acids</b>			<b>1</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>1</b>	<b>3</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>2</b>
2-Furanmethanol, acetate <sup>b</sup>	10.271	1638	-	-	-	-	-	-	0.420	-	0.395	0.040	0.255	-
Isopropyl Myristate <sup>a,b</sup>	15.961	2240	-	0.025	nd	0.020	-	0.010	-	0.030	-	0.030	-	0.020
<b>Number of esters</b>			<b>0</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>1</b>
Octanal <sup>a,b</sup>	6.450	1307	-	0.040	-	-	-	-	-	0.075	-	-	-	0.025
Nonanal <sup>b</sup>	7.946	1471	-	-	-	-	-	-	-	0.420	-	0.290	-	0.380
Furfural <sup>a,b</sup>	9.443	1576	4.710	0.500	5.860	0.870	7.125	0.790	31.235	53.435	39.645	48.520	44.885	57.735
Benzaldehyde <sup>a,b</sup>	10.131	1627	0.260	2.825	0.220	1.400	0.165	3.010	0.130	0.310	0.075	0.095	0.105	0.130
2-Propenal, 3-(2-furanyl)- <sup>a,b</sup>	14.103	2062	0.010	-	-	-	0.015	-	0.130	-	0.230	-	0.310	-
2-Furancarboxaldehyde, 5-(2-furanylmethyl)- <sup>b</sup>	18.748	2539	-	-	-	-	-	-	-	-	0.045	-	0.050	-
Benzaldehyde, 3-phenoxy- <sup>b</sup>	19.659	2644	-	-	-	-	-	-	-	-	0.045	-	0.080	-
<b>Number of aldehydes</b>			<b>3</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>3</b>	<b>5</b>	<b>4</b>
2-Heptanone, 6-methyl- <sup>a</sup>	5.915	1256	0.115	0.090	-	-	-	-	-	-	-	-	-	-
2-Octanone <sup>a</sup>	6.644	1349	0.270	1.010	-	-	-	-	-	-	-	-	-	-
2-Nonanone <sup>a,b</sup>	8.316	1495	0.750	3.210	-	0.080	0.170	0.455	0.040	-	-	0.045	-	-
2-Decanone <sup>a,b</sup>	9.623	1589	1.320	0.940	-	-	-	0.025	0.120	-	-	-	-	-
2-Undecanone <sup>a</sup>	11.112	1703	0.035	-	-	-	-	-	-	-	-	-	-	-
2-Tridecanone <sup>a</sup>	13.594	2015	0.060	-	-	-	-	-	-	-	-	-	-	-
2-Tetradecanone <sup>a</sup>	14.739	2121	0.010	-	-	-	-	-	-	-	-	-	-	-
2-Nonadecanone <sup>a</sup>	15.827	2227	0.035	-	-	-	-	-	-	-	-	-	-	-
Ethanone, 1-(2-furanyl)- <sup>a,b</sup>	9.907	1610	0.135	-	0.215	-	0.155	-	2.735	1.620	2.335	1.370	2.230	1.810
Ethanedione, di-2-furanyl- <sup>b</sup>	22.316	2953	-	-	-	-	-	-	-	-	0.030	-	0.030	-
Ethanone, 1-(4-pyridinyl)- <sup>b</sup>	13.483	2005	-	-	-	-	-	-	0.045	-	0.025	-	0.035	-
2-Propanone, 1-hydroxy- <sup>b</sup>	7.330	1430	-	-	-	-	-	-	0.140	0.085	0.030	0.145	-	0.070
2-Butanone, 4-(4-hydroxyphenyl)- <sup>a</sup>	15.387	2183	0.015	-	-	-	-	-	-	-	-	-	-	-
2-Cyclopentene-1,4-dione <sup>b</sup>	10.918	1688	-	-	-	-	-	-	0.050	-	-	-	0.030	-
1-(2-Furyl)-4,4-dimethyl-1-penten-3-one <sup>b</sup>	16.314	2276	-	-	-	-	-	-	-	-	0.005	-	0.020	-
Butyrolactone <sup>b</sup>	11.507	1735	-	-	-	-	-	-	0.140	-	0.060	-	-	-
γ-crotonolactone <sup>b</sup>	12.990	1892	-	-	-	-	-	-	0.280	-	0.140	-	0.190	-
α-angelica lactone <sup>b</sup>	8.885	1536	-	-	-	-	-	-	0.085	-	-	-	-	-
γ-octalactone <sup>a,b</sup>	14.790	2126	0.015	-	-	-	-	-	-	-	0.010	-	-	-
E-furfural acetone <sup>b</sup>	14.652	2113	-	-	-	-	-	-	0.960	-	0.780	-	0.655	-
γ-nonolactone <sup>a</sup>	15.982	2243	0.025	-	-	-	-	-	-	-	-	-	-	-
<b>Number of ketones-lactones</b>			<b>12</b>	<b>4</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>10</b>	<b>2</b>	<b>9</b>	<b>3</b>	<b>7</b>	<b>2</b>
Furan, 2-methyl- <sup>b</sup>	1.979	-	-	-	-	-	-	-	0.120	-	0.485	0.210	0.565	0.205
Furan, 2-(2-propenyl)- <sup>b</sup>	5.486	1218	-	-	-	-	-	0.030	0.035	-	0.025	-	0.015	-
Furan, 2-propyl- <sup>b</sup>	10.071	1622	-	-	-	-	-	-	0.395	0.820	0.370	0.805	0.385	-
Furan, 2-methyl-5-(methylthio)- <sup>b</sup>	8.150	1484	-	-	-	-	-	-	-	-	0.025	-	-	-
Benzofuran <sup>b</sup>	9.776	1599	-	-	-	-	-	-	-	1.135	-	0.575	-	-
2,2'-Bifuran <sup>a,b</sup>	11.040	1697	0.035	-	0.030	-	0.025	-	2.455	-	2.135	-	2.015	-
Furan, 2,2'-methylenebis- <sup>a,b</sup>	11.188	1709	-	-	-	0.085	-	-	2.125	-	2.270	-	1.915	-
Furan, 2,2'-[oxybis(methylene)]bis- <sup>b</sup>	15.434	2188	-	-	-	-	-	-	-	-	0.125	-	0.100	-
Furan, 2,2'-(1,2-ethenediyl) bis-, (E)- <sup>b</sup>	15.772	2221	-	-	-	-	-	-	1.905	-	1.335	-	1.655	-
<b>Number of furans</b>			<b>1</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>2</b>	<b>8</b>	<b>3</b>	<b>7</b>	<b>1</b>
Pyrrrole <sup>a</sup>	9.985	1616	0.095	0.275	0.085	-	0.020	0.385	-	-	-	-	-	-
1H-Pyrrrole, 1-(2-furanylmethyl)- <sup>b</sup>	13.787	2033	-	-	-	-	-	-	8.310	-	0.415	-	6.585	-
1H-Pyrrrole-2-carboxaldehyde <sup>b</sup>	15.892	2233	-	-	-	-	-	-	1.105	-	0.685	-	0.950	-
1H-Pyrrrole-2-carboxaldehyde, 1-methyl- <sup>a</sup>	11.400	1726	-	0.110	-	-	-	-	-	-	-	-	-	-
2-Pyrrolidione <sup>a,b</sup>	16.147	2259	0.105	-	-	-	0.025	-	0.005	-	-	-	-	-
2-Piperidinone <sup>a</sup>	17.111	2359	0.070	-	-	-	0.030	-	-	-	-	-	-	-
<b>Number of pyrroles</b>			<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>1</b>	<b>3</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>0</b>
Disulphide, dimethyl <sup>a,b</sup>	3.597	<600	1.390	-	2.010	-	-	-	-	-	1.070	-	-	0.025
Dimethyl trisulphide <sup>a</sup>	8.141	1484	0.480	0.115	0.495	-	0.090	0.010	-	-	-	-	-	-
Pyrazine, methyl- <sup>a,b</sup>	6.551	1329	-	0.005	-	-	-	-	-	-	-	0.005	-	0.020
Pyrazine, 2-ethyl-5-methyl- <sup>a</sup>	8.350	1498	-	0.050	0.005	-	-	-	-	-	-	-	-	-
Acetamide <sup>a</sup>	13.104	1915	0.055	0.010	0.045	0.005	0.025	-	-	-	-	-	-	-
1H-Benzotriazole, 4-methyl- <sup>a</sup>	18.351	2494	-	-	-	0.070	-	0.075	-	-	-	-	-	-
<b>Number of other compounds</b>			<b>3</b>	<b>4</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>2</b>
<b>Total number</b>			<b>25</b>	<b>16</b>	<b>11</b>	<b>9</b>	<b>16</b>	<b>10</b>	<b>11</b>	<b>14</b>	<b>8</b>	<b>13</b>	<b>8</b>	<b>12</b>

PG, respectively. In contrast, for wet gelatine samples, the level of alcohols decreased with the MR. It could be associated with the volatilization or oxidation process (Sanz et al., 2001), when heated with the xylose as reducing sugar. Alcohols in the dry gelatine samples were detected in all species at a range of 0.010 to 0.025%, while wet gelatine was detected only for BG and FG at a range of 0.090 to 0.810%.

The level of esters increased with the MR. Generally, the number and total ketones in all samples increased with the MR, except for bovine under the wet method as revealed by the generation of ketones during the Maillard reaction. According to Shahidi (1998), thermal oxidation or decomposition of Polyunsaturated Fatty Acids (PUFA), degradation of amino acids, or microbial oxidation can all generate ketones. The highest level of ketones was resulted from bovine species, except for MRPs under the wet method, whereas PGX was predominant.

In this study, a total amount of furans was found at a relatively high level. It could be related to the reactivity of xylose, which is used as reducing sugar. The main sugar-derived compounds were furans and ketones. Furans could only be formed at high temperatures (>100°C) by dehydration, fragmentation, and cyclisation of reducing sugars (Lee et al., 2012). In contrast, the ketones generally produced at a lower temperature such as 80°C (Liu et al., 2015).

Total pyrroles in samples under the dry method increased slightly after Maillard reaction (Table 1), indicating the pyrroles formation during the reaction. Total pyrroles increased by about 35 fold, 13 fold, 100 fold for BG, FG and PG under the dry method, respectively. In the MR process, pyrroles could be derived from sugar fragmentation or Strecker degradation (Van Boekel, 2006). Heat application, such as extrusion cooking and microwave heating, also produced pyrrole derivatives (Nollet et al., 2012). The highest pyrroles were generated by bovine-MRPs, while the lowest was generated by fish-MRPs. In contrast, no pyrroles were detected in the wet-MRPs for all species and low levels of pyrroles were detected for BG and PG in the wet samples. This result could be generated by the low concentration of samples.

Table 2 represents in detail the VCs which were detected in gelatine and the MRPs. Among them, 38 compounds were found in gelatine, 47 compounds were found in the MRPs and 18 compounds were found in both. Eight alcohols were detected in gelatine and the MRPs samples. The numbers of alcohols in the MRPs samples under the dry method were predominant (See Table 2). Of these substances, 2-furan methanol, 3-pyridinol, 1,4-benzenediol and 2-methyl- were the common components of the dry-MRPs from all species. However, 3-pyridinol was not detected from BGX samples. Linear alcohols, namely 1-heptanol and 1-hexanol, 2-ethyl were detected in BG and FG samples under the wet method, which are derived from the branched aldehydes reduction (Domínguez et al., 2019).

Eight acids were detected in the samples; 4 of which were detected for bovine and fish samples, whereas all of which were found in the porcine samples (Table 2). Among these acids,

acetic acid was detected predominantly in the samples, except for BG samples under the wet method. From the analysis, the highest acetic acid contents were generated by samples from fish (FG and FGX), from both methods. In contrast, the lowest acetic acids for gelatine were generated by BG under dry method at 0.080% of relative content. Next, the lowest acetic acid for the MRPs was generated by PGX under wet method, with the relative content of 0.145%. Propanoic acid, hexanoic acid and octanoic acid were obtained from mammal samples. Propanoic acid was only detected from PG under the dry method. In addition, hexanoic acid and octanoic acid were found only in BGX and PGX under the wet method. In contrast, butanoic acid was only detected in dry-porcine gelatine samples, while dodecanoic acid, tetradecanoic acid and n-hexadecanoic acid were detected only in porcine with dry-MRPs samples.

According to Domínguez et al. (2019), the low-chain organic acids have a significant effect on the aroma characteristics of meat products. Acetic acid is related to sour, vinegar, and pungent aroma (Malgorzata et al., 2016). Propanoic acid generates pungent, fruity and cheesy aroma (Peinado et al., 2016). Butanoic acid contributes to sweet and cheesy aroma (Domínguez et al., 2019; Feng et al., 2015; Mahmoud and Buettner, 2017), while hexanoic, octanoic and dodecanoic acids result in musty and pungent aroma (Domínguez et al., 2019; Mahmoud and Buettner, 2017).

Only two esters were detected, namely 2-furanmethanol, acetate and isopropyl myristate. 2-furanmethanol and acetate were detected only for dry-MRPs, and were related with roast and medicinal aroma, sweet, fruity and banana odour (Yang et al., 2012). On the other hand, isopropyl myristate were detected for both gelatine and MRPs under wet method, related to faint, oily and fatty odour. Since their odour strength were low, they have no significant effect on the overall aroma.

A total seven aldehydes were detected for the samples, and five aldehydes were detected for dry-method, while four aldehydes were generated by the samples under wet method, as shown in Table 2. In this study, furfural was the predominant compound in the aldehydes group, with relative content at the range of 0.500-7.125% for gelatine samples. The level of furfural increased by 6 to 21 times when subjected to the MR with the range of 31.235-57.736% for the MRPs samples. In contrast, the level of benzaldehydes in the samples decreased when subjected to the MR, especially for the samples under wet method. It could be related to the heterocyclisation process in the MR.

Two linear aldehydes, namely octanal and nonanal were detected for the samples under wet method. Octanal was detected for BG, BGX, and PGX, while nonanal was identified in the wet-MRPs from all species. Only one branched aldehyde, namely 2-propenal, 3-(2-furanyl)- was generated by all species, except in FG. In addition, four cyclo-aldehydes, such as furfural, benzaldehydes, 2-furancarboxaldehyde, 5-(2-furanmethyl)- and benzaldehyde, 3-phenoxy- were detected. Furfural and benzaldehyde were detected for all samples from all species, both

in the dry and wet method, while 2-furancarboxaldehyde, 5-(2-furanmethyl)- and benzaldehyde, 3-phenoxy- were detected for FGX and PGX samples under the dry method. Branching aldehydes, according to Domínguez et al. (2019), are involved in proteolysis and amino acid breakdown. Furfural comes from Amadori rearrangement, while benzaldehyde is produced from Strecker degradation of certain amino acids, such as leucine and phenylalanine (Chen et al., 2019; Domínguez et al., 2019).

Aldehydes generally have a lower aroma threshold than alcohols (Fu et al., 2020); therefore, they have a great contribution to the overall aroma and even the low contents of aldehydes. Octanal and nonanal are related with fat, sweet and green aroma. Octanal contributes to the meat-like, green, fresh, grass, and fruity notes, while nonanal imparts a sweet and fruity aroma (Domínguez et al., 2019). Furfural contributes to sweet, almond and baked aroma (Feng et al., 2015; Ong et al., 2015), while benzaldehyde gives burnt sugar and almond-like aroma (Gong et al., 2017; Liu et al., 2015).

A total of 21 compounds were generated by the ketones group (Table 2). Most of them were found in the MRPs samples under the dry method, especially for bovine samples, whereas 14 compounds were found in BG samples and 11 compounds were resulted from BGX samples. Only six compounds from the ketones group were detected from samples under the wet method, namely ethanone, 1-(2-furanyl)-, 2-heptanone, 6-methyl-, 2-octanone, 2-propanone, 1-hydroxy-, 2-nonanone, 2-decanone. Ethanone, 1-(2-furanyl)- was detected in all species samples, except in gelatine under the wet method. It contributes to sweet, balsam, almond, cocoa, coffee and caramel-like aroma. Also, five lactone compounds, namely butyrolactone,  $\gamma$ -crotonolactone,  $\alpha$ -angelicalactone,  $\gamma$ -octalactone and  $\gamma$ -nonalactone were detected in dry BG, dry BGX, and dry FGX samples. Generally, lactones come from  $\beta$ -oxidation of oleic and linoleic acids (Mahmoud and Buettner, 2017). However, MR can also promote lactones (Domínguez et al., 2019). The lactones are contributing to the overall aroma of MRPs with a pleasant, buttery, fatty, creamy, fruity and coconut-like odour (Mahmoud and Buettner, 2017).

Nine furans were detected from all samples, mainly found in MRPs samples under dry method with a similar level. Furans were commonly identified in the cooked meat and were considered could exhibit some cooked flavour (Liu et al., 2015). Generally, furans could not contribute to sample odour due to their quite high odour threshold values (Su et al., 2011).

There were six compounds in the pyrroles group, as shown in Table 2. Among pyrroles, 1H-pyrrole and 1-(2-furanylmethyl)- were predominant. Two proline-specific MRPs were also detected, namely pyrrolidinone and piperidinone. They were resulted from the interaction between proline  $\alpha$ -dicarbonyl. In this study, pyrrolidine and piperidinone were detected in dry mammal gelatine. Pyrrole and pyrrole derivatives are associated with burnt and earthy aromas (Hou et al., 2017). Pyrrolidines can be associated with cereal-like aroma, while piperidinone does not have a specific characteristic aroma (Ikan, 1996).

In the other groups, sulphur-containing compounds, pyrazines, acetamide and oxazole were detected. Two sulphur-containing compounds, namely dimethyl disulphide and dimethyl trisulphide, were identified in this study. Sulphur-containing compounds are known to be generated from methionine and cysteine amino acids. Methionine is the main precursor of volatile sulphur-containing compounds. Methionine is converted to methional by Strecker degradation, which serves as a further oxidized form of methanethiol. Dimethyl disulphide and dimethyl trisulphide arise from the oxidation of methanethiol (Mishra et al., 2017). Of the sulphur-containing compounds, dimethyl disulphide was the most predominant. It was detected mainly from bovine and fish gelatine for the dry and wet methods, and in porcine MRPs under the wet method at a relatively low level. In contrast, dimethyl trisulphide was only detected in samples under the dry method. Dimethyl disulphide and dimethyl trisulphide may lead to sulphur, roasted and meaty flavour (Chiang et al., 2019; Van Boeckel, 2006). Their aroma contributes significantly to the overall aroma because of their low odour threshold.

Pyrazine is known as the common compound found in the heated product. In this study, two pyrazines, namely methyl pyrazine and 2-ethyl-5-methyl pyrazine were detected at a very low level. Methyl pyrazine was generated by all species of MRPs, while 2-methyl-5-methyl-pyrazine was detected from FG and BGX samples under the dry method. Two nitrogen atoms in pyrazines ring structure could be derived from amino nitrogen in amino acids, peptides, and free ammonia released. It can be produced from serine and threonine pyrolysis (Mishra et al., 2017) and from MR. The interaction of  $\alpha$ -amino acids and reducing sugars initially generates Amadori and Heyns products, and rearrangement of those products leads to the formation of furfurals or reductones, including  $\alpha$ -dicarbonyls. Furthermore, Strecker degradation converts  $\alpha$ -dicarbonyls into  $\alpha$ -amino carbonyls, which finally condenses to form pyrazines. Moreover, because sugar degradation also produces  $\alpha$ -dicarbonyls, Strecker degradation could directly produce pyrazines. During the MR, free ammonia produced by deamidation and deamination can be involved in the formation of pyrazines (Lee et al., 2012). Pyrazines have low odour thresholds, and may play a significant impact in the scent of various cooked food (Ikan, 1996). Alkyl-pyrazine could be related to a nutty-like odour (Mishra et al., 2017).

In addition, acetamide was detected in all species under the dry method except in porcine-MRPs. Acetamide is the simplest amide derived from acetic and free ammonia. In this system, it was found as an Amadori product (Yang et al., 2012). Also, 1H-benzotriazole, 4-methyl- as the product of Strecker degradation was generated by fish and porcine-MRP samples under the dry method at a relatively similar level.

Based on Table 2, the five most abundant VCs in the gelatine were furfural (sweet aroma), acetic acid (sour and pungent aroma), nonanone (floral and fruity), dimethyl disulphide (onion, cooked cabbage-like aroma), and decanone (orange-like, musty and sweet aroma). Additionally, the most abun-

dant MRPs were furfural, 1-(2-furanylmethyl)-1H-pyrrole (mushroom-like aroma), 1-(2-furanyl)-ethanone (balsamic), acetic acid and 2,2'-bifuran. However, according to Feng et al. (2015), beside concentration, Odour Threshold (OT) influences the ultimate contribution of a certain compounds to the overall aroma. Of all the identified VCs, seven VCs may also contribute to the overall aroma of samples due to their low OT. The VCs include heptanol (musty and green aroma, OT = 3 µg/kg), octanal (citrusy, OT = 0.700 µg/kg), nonanal (green and citrusy, OT = 1 µg/kg), 2-octanone (herb and butter-like aroma, OT = 50 µg/kg), 2-nonanone (floral and fruity, OT = 200 µg/kg), dimethyl disulphide (onion, cooked cabbage-like aroma, OT = 1.100 µg/kg) and dimethyl trisulphide (cooked onion-like aroma, OT = 0.100 µg/kg). Therefore, they also became the major contributors to the overall aroma of gelatine and the MRPs.

#### 4. CONCLUSION

The predominant VCs which are responsible for the aroma of gelatine and the MRPs were investigated using HS-SPME-GCMS. Furfural, acetic acid, nonanone, dimethyl disulphide, and decanone were detected as the abundant volatiles in gelatine. On the other hand, furfural, 1-(2-furanylmethyl)-1H-pyrrole, 1-(2-furanyl)-ethanone, acetic acid and 2,2'-bifuran were predominant in the MRPs samples. Five volatile compounds with low odour threshold were also detected, namely heptanol, octanal, nonanal, nonanone and dimethyl trisulphide. All these volatile compounds gave significant contribution to the overall aroma of samples. Therefore, they could be suggested as important compounds in differentiation the sources of gelatine. Of all the important VCs, bovine gelatine could be characterised from nonanone, decanone, 1-2-furanyl methyl-1H pyrrole, 1-2-furanyl-ethanone, heptanol, octanal and octanone. Furthermore, fish gelatine could be characterised from acetic acid, dimethyl disulphide and dimethyl trisulphide, while porcine gelatine from furfural, 1-2-furanyl methyl-1H pyrrole and nonanal.

#### 5. ACKNOWLEDGEMENT

This research supported by research funding from Indonesia Endowment Fund for Education (LPDP[PRJ-133/LPDP.3/2017].

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