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Porcine, bovine, and mixed gelatin identification using SPME-GC-MS and chemometrics

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A B S T R A C T

Gelatin is a versatile raw material extensively used in the food, cosmetics, and pharmaceutical industries. It is produced globally by partially hydrolyzing collagen derived from pigs and cows, leading to religious and ethical concerns among various communities. Therefore, this study aimed to explore alternative methods to distinguish porcine, bovine, and mixed gelatin by analyzing the unique profiles of their total volatile compounds. The volatilomics method integrated solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) with chemometrics. The results showed that principal component analysis (PCA) of the volatile compounds from gelatin powder had clear classification among porcine, bovine, and mixed gelatin, suggesting the discrimination ability of the method. Furthermore, partial least squares discriminant analysis (PLS-DA) identified distinct marker compounds that significantly contributed to the classification of each gelatin type. The marker compounds for porcine gelatin included 2-decen-1-ol, 2-dodecenal, cyclohexane 1-butenylidene, decane 3,6-dimethyl, cyclohexanone 2-propyl, borinic acid, 3-tetradecyn-1-ol, 2-tridecene, 5,5-dimethyl-1,3-dioxan-2-one, and 2-n-butyl furan. For bovine gelatin, the marker compounds were 2-heptanone 3-methyl, nonane 5-butyl, tridecane 6-methyl, 1-hexacosanol, nonane 3-methyl-5-propyl, undecane 3-methyl, octane 4-methyl, 2,4-undecadienol, and 1-hexadecanol 2-methyl.

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1 Introduction

Gelatin is a high molecular weight polypeptide made from collagen of skin, bones, and connective tissues of various animals, such as cattle, pigs, fish, and poultry (Gomez-Guillen *et al.* 2011; Jannat *et al.* 2018) The most common sources include pork skin (46%), bovine hide (29.4%), with pork and cattle bones (23.1%) (Gomez-Guillen *et al.* 2011). Due to its ability to thicken and gel, gelatin has been used in the food and pharmaceutical industries as a stabilizer in food or to produce soft and hard capsules, wound dressings, and adsorbent pads (Widyasari & Rawdkuen 2014; Hassan *et al.* 2018). Generally, Muslim communities based on their religious preference do not consume porcine-based gelatin, showing the need for a method capable of differentiating pork components. The most widely used porcine component differentiation test methods have been DNA-based analysis using PCR (Gina *et al.* 2024) and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (Salamah *et al.* 2019). However, both methods have drawbacks in the analysis process such as the dependence of PCR on specific DNA in gelatin. This is because DNA may not be detected when the raw molecules are denatured during acid/base treatment with high temperature gelatin production process (Sudjadi *et al.* 2016). The LC-MS/MS method is also highly complicated, requiring a long completion time to complete, specific peptides derived from protein cleavage by enzymes as marker, and is significantly expensive (Salamah *et al.* 2019). Therefore, a new method that is faster, less expensive, and easier to differentiate the origin of gelatin is highly needed. One potential authentication method is using additional gelatin properties, which are different sources with unique aroma and volatilomes.

Volatilomes are terms used to represent all volatile compounds found in a biological species, environment, or material including those formed by microbial metabolic processes and derivatives from exogenous sources (Lytou *et al.* 2019; Casaburi *et al.* 2015; Watanabe *et al.* 2015). Gelatin possessed a unique aroma of volatile compounds derived from raw materials trapped during the manufacturing process. This unique aroma can serve as marker for differentiating pork components in gelatin based on the profiles of volatile compounds. Several studies have used solid-phase microextraction (SPME) method in conjunction with gas chromatography-mass spectrometry (GC-MS) such as profiling the physicochemical properties and odor of gelatin produced from seabass (*Lates calcarifer*) skin (Sae-Leaw & Benjakul 2015), discriminating meatballs (beef, chicken, wild boar, and mixtures) (Pranata *et al.* 2021), along with analyzing spoilage minced beef stored in various packaging and temperature conditions (Argyri *et al.* 2015).

This study aimed to explore alternative methods to distinguish porcine, bovine, and mixed gelatin by analyzing the unique profiles of their volatile compounds. The differential in these volatile compounds are essential premise for recognizing species-specific gelatin sources. The SPME has the benefit of being rapid, simple, and useful in studying a wide range of metabolites in various matrices (Lin *et al.* 2012; Reyes-Garcés & Gionfriddo 2019). Furthermore, the GC-MS is a selective and sensitive metabolomic profiling method that produces complicated datasets for statistical methods such as multivariate analysis to process large amounts of data. In this study, multivariate analysis was considered ideal for comparing complex spectrum data, finding patterns in compounds, and determining chemical compositional variations across samples (Maree *et al.* 2014).

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2 Materials and Methods

2.1 Materials

Samples of standard porcine and bovine gelatin were obtained from Sigma Aldrich, while commercial bovine was acquired from commercial market in Bogor, West Java, Indonesia. GC-MS QP2020 NX (Shimadzu) was combined with the SPME fiber assembly divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco). SIMCA-P software version 16 (Umetrics, Umea, Sweden) was used for multivariate analysis.

2.2 Sample Preparation and Extraction with SPME

Initially, gelatin samples were pulverized using a mortar and pestle. The mashed gelatin sample was weighed at 2 grams and placed in a 20 mL vial, followed by the addition of 5 mL distilled water and 0.5 μL ethanol, which was mixed homogeneously. Pre-extraction was carried out for 3 hours at 80℃ using a plate heater and SPME fiber was placed in the headspace for 50 minutes at 80℃ for extraction. Each sample received the same procedure and repeated it 5 times.

2.3 Analysis of Volatile Compounds by GC-MS

The materials used for the experiment conducted in this research were divided into samples and reagents. The samples consisted of various meats and meat products, including pork, beef, goat, chicken, duck, salmon,GC-MS analysis was conducted according to a previous study (Sae-Leaw & Benjakul 2015) with slight modifications. Specifically, GC-MS QP2020 NX was used to analyze volatile compounds and Helium (He) was applied as the carrier gas, with a constant flow rate of 1 mL/min. The injection port operated in splitless mode at 250℃, while a Stabilwax capillary column (60 m x 0.25 mm, film thickness 0.25 m) was used to separate the compounds. For the first minute, the oven temperature was set to 35℃ and increased to 70℃ at a rate of 5℃ per minute. There was a continuous increase to 170℃ at a rate of 10℃/min and held for 7 minutes. The temperature reached 250℃ at 3℃/min with the interface fixed at 280℃. The mass spectrometer was configured to electron ionization (EI) mode with a scan range of 25–500 m/z and electron energy of 70 eV. The temperature of the MS source (ion source) was fixed at 240℃ and SPME fiber was introduced into the GC-MS injection port, which was worked for 5 minutes. The analysis was initiated by clicking the start button on the GC-MS, which lasted for 51.67 minutes to complete, with each sample receiving 5 injections.

2.4 GC-MS Data Processing and Identification of Volatile Compounds

The peak processing parameters were selected as follows: total peak = 500, slope = 100/min, width = 1 sec, and minimum area = 0. The mass spectral matching was performed automatically with a minimum similarity of 80%. The alleged compounds identified with the Shimadzu built-in software were confirmed by calculating the retention index (RI) value. The homologous n-alkane series (C_{10}-C_{36}) was used to strengthen the alleged compounds with the difference in the RI value allowed ±20. The eluting procedure was carried out under the same chromatographic conditions. As a reference, the RI value was compared to the NIST 17 database. The RI value can be calculated using the Equation 1 (Wang *et al.* 2017):

$$RI(x) = 100Z + \frac{TR(x) - TR(z)}{TR(z+1) - TR(z)} \quad \text{..... (1)}$$

where: RI(x): the unknown compound's retention index, z: the number of carbon atoms of the *n*-alkane eluted before the unknown compound x, z + 1: the number of carbon atoms of the *n*-alkane eluted after the unknown compound x, TR(x): the retention time of each volatile compound x, TR(z): the retention time of the *n*-alkane eluting directly before compound x, and TR(z + 1): the retention time of the *n*-alkane eluting directly after compound x.

2.5 Multivariate Analysis

The data matrix was initially processed using principal component analysis (PCA) to discover the classification pattern of different samples. The PCA data was expected to be classified into 4 categories comprising samples of porcine, standard bovine, commercial bovine, and mixed gelatin. Furthermore, the performance of the PCA model was assessed using R^2X and Q^2 values, with $Q^2 > 0.5$ (Belasco *et al.* 2015). The classification pattern that was developed was refined using partial least squares discriminant analysis (PLS-DA). The values of R^2Y and Q^2, which were both in the region of 0.5-1, showed the correctness of the PLS-DA model (Eriksson *et al.* 2006). Subsequently, the permutations test and CV-ANOVA test were used to confirm that the model created was genuine without overfitting.

3 Result

3.1 Profile of Volatile Compounds using GC-MS

The highest concentration of compounds found in the porcine gelatin samples came from alcohols, cyclic hydrocarbons, and ketones, based on the intensity of the broad peak area observed in each sample. This was consistent with previous studies where aldehyde, ketone, and hydrocarbon groups had the highest peak area intensity in pork meat samples (Meinert *et al.* 2007). Ketone compounds, aliphatic hydrocarbons, and alcohols were the highest volatile compounds for the standard and commercial bovine gelatin samples. It was also reported that aldehyde, ketone, and hydrocarbon groups had the highest peak area intensity in beef samples (Wang *et al.* 2017). The largest concentrations of volatile compounds were found in mixed gelatin samples of aliphatic hydrocarbons, alcohols, and aldehydes, as shown in Figure 1.

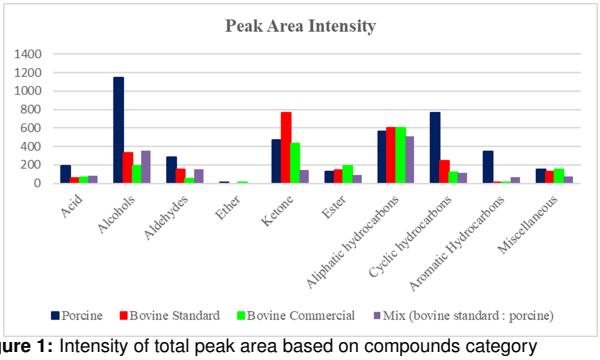

Figure 1: Intensity of total peak area based on compounds category

3.2 Classification of Gelatin Samples with Multivariate Analysis

Classification of gelatin started with creating a dataset including all samples using the area data for each identified compound. Subsequently, the values of all detected compounds were normalized by dividing the area of each compound by the total area. The model was constructed using both unsupervised (PCA) and supervised (PLS-DA) methods. A multiplicative signal correction (MSC) data filter was applied to reduce noise in the data. The PCA analysis, with 3 principal components (PC), showed an overall data variance (R^2X) of 0.571 and a Q^2 value of 0.328. Typically, an R^2X and Q^2 value greater than 0.5 indicates a robust PCA model. Although the Q^2 value in this study was below 0.5, the sample grouping was distinctly observable (Figure 2).

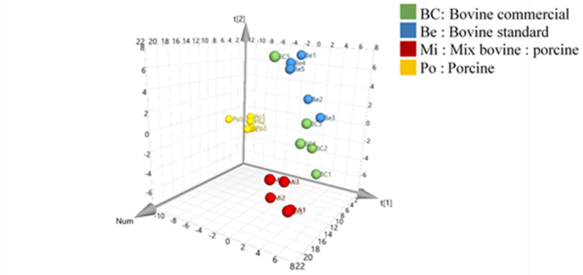

Figure 2: Unsupervised score plot (UV scaling, multiplicative signal correction filter, 2 principal components); R^2X = 0.571 and Q^2 = 0.328

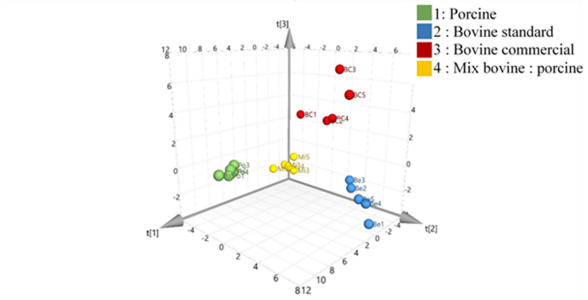

Figure 3: Supervised multivariate analysis 3D score plot of 4 classes; R^2Y = 0.741 and Q^2 = 0.932

A supervised multivariate analysis (PLS-DA) was performed, showing that the overall cumulative variance of data (R^2Y) of 0.741 and Q^2 value of 0.932 with 3 PC and 4 classes could explain 95 variables. Generally, an appropriate model requires R^2Y and Q^2 values in the 0.5-1 range (Eriksson et al. 2006). The classification pattern was shown in the PLS-DA 3D score plot compared to the PCA 3D score plot, as presented in Figure 3. The gelatin samples were divided into 4 classes based on the 3D PLS-DA score plot. The porcine gelatin samples were grouped

and placed in the bottom left of the classes, showing a good classification pattern. Meanwhile, commercial and standard bovine gelatin samples were successfully distinguished.

Due to differences in volatile compounds, the 4 classes of PLS-DA model could categorize and separate each class of gelatin samples, including commercial bovine gelatin and mixed samples. The porcine gelatin sample plotted away from the bovine suggested that volatile compounds could be used to distinguish the authentication of gelatin halal. The mixed sample was separate from others in the score plot, showing the effectiveness of the method to detect gelatin adulteration. The volatile compounds contributing to the classification of the 4 classes were shown in the PLS-DA loading biplot, as presented in Figure 4. The responsible volatile compounds in each class were discovered in the same area as the sample code on the PLS-DA score plot. Some compounds contributing to the porcine gelatin (class 1) were 2-decen-1-ol, 2-dodecenal, cyclohexane 1-butenylidene, decane 3,6-dimethyl, cyclohexanone 2-propyl, borinic acid, 3-tetradecyn-1 -ol, 2-tridecene, 5,5-dimethyl-1,3-dioxan-z-one, and 2-n-butyl furan. Standard bovine gelatin (class 2) contained compounds 2-heptanone 3-methyl, nonane 5-butyl, tridecane 6-methyl, 1-hexacosanol, nonane 3-methyl-5-propyl, undecane 3-methyl, octane 4-methyl, 2,4-undecadienol, and 1-hexadecanol 2-methyl. Meanwhile, commercial bovine gelatin (class 3) contained compounds dodecane 2-methyl-6-propyl, octane 2,2-dimethyl, nonane 2,5-dimethyl, hexadecane, docosanoic acid, benzene 1-isocyano-z-methyl, pentadecane 7-methyl, 1,2-oxathiane 6-dodecyl, octacosyl trifluoroacetate, 2-bromo dodecane. The mixed gelatin (class 4) included 2,4-decadienal compounds, 5-methyl undecane, 2-decanoic, eicosanal, 1-octanol 2-butyl, tridecane 2-methyl, and 1 nonadecene.

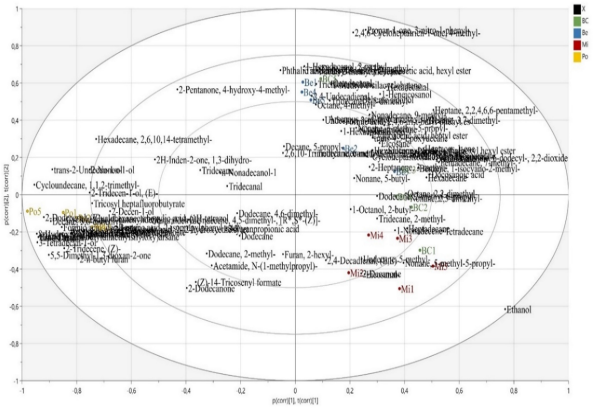

Figure 4: Supervised multivariate analysis loading bi plot of 4 classes; porcine gelatin (1), standard bovine gelatin (2), commercial bovine gelatin (3), and mixed gelatin (4)

3.3 Identification of Marker Compounds

The volatile compounds that played a role as markers in each class were identified using coefficient and VIP (Variable Influence on Projection) plots. Specifically, VIP plots showed volatile compounds influence on grouping (Sri Harsha *et al.* 2018), with positive coefficients suggesting dominance or most abundant compounds, while negative indicated only minimally present compounds in the sample. Based on the results, 5 compounds having positive and negative correlations with high VIP values (>0.5) were selected, as shown in Table 1.

4 Discussion

4.1 Profile of Volatile Compounds using GC-MS

The analysis of volatile compounds in standard bovine gelatin, standard porcine, commercial bovine, and mixed (1:1) samples formed during the heating process obtained chromatograms with intensity and different peak areas for each sample. The amount of analyte extracted by SPME was also affected by SPME fiber type, extraction temperature, extraction time, and the number of samples used (Schmidt & Podmore 2015). Based on the identification of volatile compound profiles, approximately 156 compounds representing all samples were obtained. Meanwhile, volatile compounds in porcine gelatin, standard bovine gelatin, commercial bovine gelatin, and mixed gelatin were obtained 73, 77, 84, and 80, respectively. Volatile compounds that have been identified can be divided into 10 classes based on their functional groups, namely acids, alcohols, aldehydes, ketones, ethers, esters, aliphatic hydrocarbons, aromatic hydrocarbons, cyclic hydrocarbons, and miscellaneous.

Compounds derived from group alcohol, aldehydes, ketones, esters, hydrocarbons, terpenes, and other substances were identified as volatile in the bovine and porcine samples (Pavlidis *et al.* 2019). The sample type, chemical content, and processing method determined the types and compositions of volatile compounds produced. Thermal degradation of protein, enzymatic reactions, lipid oxidation, decarboxylation reactions, and Maillard reactions were all reactions that produced volatile compounds (Kosowska *et al.* 2017). Based on grouping and the type of gelatin sample, the peak area and categorization were combined to identify the dominating group of compounds in each gelatin sample.

Aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acids, and esters are among the volatile compounds generated from the lipid degradation pathway. As the primary lipid degradation products, aldehydes are most implicated in the aroma characteristics of specific species (Mottram 1998). Saturated and unsaturated aldehydes, which had 6 to 10 carbon atoms, played a significant part in aroma formation (Kosowska *et al.* 2017). The most common aldehyde detected in porcine gelatin was determined by compound (E)-2-dodecenal, followed by tridecanal and glutaraldehyde. The group compounds of aldehydes in porcine gelatin were found to be 2,2-dimethylocta-3,4-dienal and cis-4-decenal, while commercial bovine gelatin contained pentadecanal and henicosanal. Compounds heptanal, (E-E)-2,4-nonadienal, tetradecanal, and 2-bromooctadecanal were detected in mixed gelatin, while (E,E)-2,4-decadienal was discovered in porcine and mixed gelatin. According to Chen *et al.* (2019), (E,E)-2,4-decadienal is the main volatile component in pork that contributes to flavor.

Table 1: Compounds having positive and negative coefficient values with the highest variable influence on projection values from each class

Class	Compound	Coefficient	VIP
Porcine gelatin	**Positive compounds**		
	1. 2-n-butyl furan	0.052069	2.04887
	2. 3-Tetradecyn-1-ol	0.051501	1.77337
	3. Cyclohexane, 1-butenylidene-	0.045176	1.60645
	4. trans-2-undecen-1-ol	0.036048	1.58739
	5. 5,5-Dimethyl-1,3-dioxan-2-one	0.032791	1.45484
	Negative compounds		
	1. Sulfurous acid, 2-ethylhexyl hexyl ester	-0.000447	2.09524
	2. Hexadecane, 1-iodo-	-0.000648	1.63011
	3. Formic acid, 2,4,6-tri-t-butyl-phenyl ester	-0.006666	1.48650
	4. Hexadecane	-0.008486	1.22675
	5. Heptadecane	-0.002901	1.15589
Standard bovine gelatin	**Positive compounds**		
	1. Propan-1-one, 3-nitro-1-phenyl-	0.169044	3.77241
	2. Pentadecane, 7-methyl-	0.043914	1.81357
	3. Cycloundecane, 1,1,2-trimethyl-	0.022607	1.40681
	4. 1-Iodo-2-methylundecane	0.058797	1.18117
	5. Phthalic acid, butyl tridec-2-yn-1-yl ester	0.053315	1.14638
	Negative compounds		
	1. Ethanol	-0.041841	4.20916
	2. Sulfurous acid, 2-ethylhexyl hexyl ester	-0.060209	2.09524
	3. Formic acid, 2,4,6-tri-t-butyl-phenyl ester	-0.030609	1.77337
	4. Hexadecane, 1-iodo-	-0.047339	1.63011
	5. 2-n-butyl furan	-0.043235	1.63011
Commercial bovine gelatin	**Positive compounds**		
	1. 1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	0.098270	2.26567
	2. Sulfurous acid, 2-ethylhexyl hexyl ester	0.114092	2.09524
	3. Hexadecane, 1-iodo-	0.088604	1.63011
	4. Docosanoic acid	0.021654	1.25714
	5. Hexadecane	0.064359	1.22675
	Negative compounds		
	1. Ethanol	-0.032702	4.20916
	2. Propan-1-one,3-nitro-1-phenyl-	-0.036964	3.77241
	3. 2-n-butyl furan	-0.030652	2.04887
	4. Pentadecane, 7-methyl-	-0.083003	1.81357
	5. 3-Tetradecyn-1-ol	-0.005329	1.77337
Mixed gelatin	**Positive compounds**		
	1. Ethanol	0.123849	4.20916
	2. 2-n-butyl furan	0.021818	2.04887
	3. Pentadecane, 7-methyl-	0.073021	1.81357
	4. 5,5-Dimethyl-1,3-dioxan -2-one	0.018877	1.45484
	5. Docosanoic acid	0.054738	1.25714
	Negative compounds		
	1. Propan-1-one, 3-nitro-1-phenyl-	-0.081212	3.77241
	2. 1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	-0.101076	2.26567
	3. Sulfurous acid, 2-ethylhexyl hexyl ester	-0.053435	2.09524
	4. Hexadecane, 1-iodo-	-0.040617	1.63011
	5. Cyclohexane, 1-butenylidene-	-0.014472	1.60645

4.2 Classification of Gelatin Samples with Multivariate Analysis

The PCA score plot (Figure 2) showed that the classification pattern of porcine gelatin samples was distinguishable from bovine gelatin (both commercial and standard), as well as mixed from bovine and porcine. However, a significant difference was observed between the commercial and standard bovine samples due to variations in bovine breeds or the components of bovine (skin or bones) used in gelatin manufacturing, which led to distinguished volatile compound profiles. According to Zafeiropoulou *et al.* (2012), the release of aroma compounds from the gelatin matrix was influenced by several parameters, namely origin, processing, mechanical features of the resulting, and volatile properties. Diversity in volatile compounds could also be produced by differences in the raw materials conditions before gelatin processing, as proven in the study on the increased aldehyde and alcohol influence fishy aroma in gelatin samples (Sae-Leaw & Benjakul 2015). These variations were caused by fish skin being frozen for longer before processing into gelatin, suggesting potential occurrence in other animals such as bovine. However, no tests were conducted to verify the results, showing the need for further investigations.

Porcine gelatin was found to contain the highest levels of aldehyde compounds. Aldehydes and furans are significantly present in pork and are essential components of its flavor profile (Chen *et al.* 2019). Specifically, 2-butylfuran was the only compound identified in both porcine and mixed gelatin. Alcohol compounds, similar to aldehydes, are produced through lipid oxidation pathways. When heating cysteine, ribose, and lecithin, alcohols, and alkylfurans could form as substitutes for aldehydes (Farmer & Mottram 1992). The most common alcohol compounds identified across the 4 sample groups were trans-2-undecen-1-ol, nonadecan-1-ol, and docosan-1-ol, with porcine gelatin showing the highest abundance. Additionally, compounds such as 3-tetradecyn-1-ol, 9-decen-1-ol, heptadecan-1-ol, and 2-methyldecan-1-ol were found in porcine or mixed gelatin. Regarding acidic groups, erucic and diethylborinic acids were unique to porcine gelatin, while tricosanoic and docosanoic acids characterized bovine and absent in mixed gelatin.

The classification pattern PLS-DA model (Figure 4) was better compared to the model PCA based on values R^2Y and Q^2. However, 100 random permutations were applied to validate that the model obtained was correct without the occurrence of overfitting, as shown in Figure 5. According to the permutation test results, the R^2Y and Q^2 values obtained from the permutation analysis in classes 1, 2, and 4 were lower than the original R^2Y and Q^2 values. In class 3, there was appearance of overfitting because the permuted R^2Y value was identical to the original R^2Y model. Overall, the model PLS-DA obtained was correct and no overfitting occurred. The PLS-DA model was further validated using CV-ANOVA, which was used to measure the level of dependability. The p-value is among the most significant factors in determining the model reliability in the CV-ANOVA test procedure. Based on the results, p-value obtained in the investigation was less than 0.05, indicating that the model was reliable.

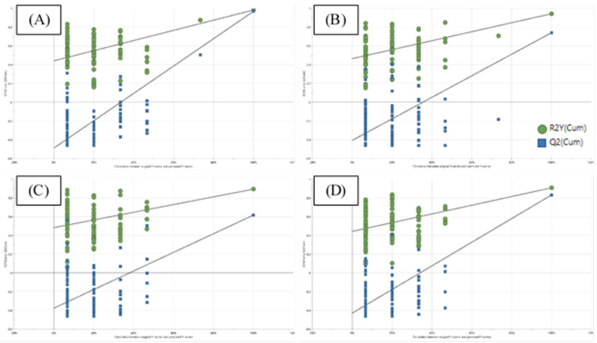

Figure 5: Supervised multivariate analysis model permutation test; class 1 (A), class 2 (B), class 3 (C), and class 4 (D)

4.3 Identification of Marker Compounds

Table 1 shows that the compounds 2-n-butyl furan, 3-tetradecyn-1-ol, cyclohexane, 1-butenylidene-, trans-2-undecen-1-ol, and 5,5-dimethyl-1,3-dioxan-2-one were volatile with the highest VIP values in the porcine gelatin (class 1). Previously, no studies identified marker compounds in porcine and bovine gelatin, but there were investigations on the origins of gelatin raw materials. According to a previous study (Zhang *et al.* 2017), 2-pentyl-furan was a volatile compound identified in pork bone soup with a "meaty and sulfurous" aroma. Meanwhile, the aroma of 2-n-butyl furan had not been determined and trans-2-undecen-1-ol was discovered in cooked pork (Yang *et al.* 2014). There were no investigations on the presence of 3-tetradecyn-1-ol, cyclohexane, 1-butenylidene, and 5,5-dimethyl-1,3-dioxan-2-one in porcine gelatin or raw materials.

The volatile compounds with the highest VIP values in standard bovine gelatin (class 2) included propan-1-one 3-nitro-1-phenyl-, pentadecane 7-methyl-, cycloundecane 1,1,2-trimethyl-, 1-iodo-2-methylundecane, and phthalic acid butyl tridec-2-yn-1-yl ester. For commercial bovine gelatin (class 3), the compounds were 1,2-oxathiane, 6-dodecyl-, 2,2-dioxide, sulfurous acid, 2-ethylhexyl hexyl ester, hexadecane, 1-iodo-, docosanoic acid, and hexadecane. Currently, there have been no reports identifying these compounds in bovine gelatin or its raw materials. However, 1-iodo-2-methylundecane has been detected in herbal formulations with reported antimicrobial activity. Phthalic acid butyl tridec-2-yn-1-yl ester, a volatile component found in fermented foods was also reported for antibacterial properties.

Compounds 1,2-oxathiane, 6-dodecyl-, 2,2-dioxide was reported in Alstonia boonei leaf oil (Okwu & Ighodaro 2010), 3,7-dimethyldecane in fresh beef (Bhattacharjee *et al.* 2011), and 5-methyltetradecane in beef (Shahidi *et al.* 2009). A previous study also found that hexyl 2-cyanoacetate, a hexyl ethanoate with a sweet-smelling ester group, was discovered in beef (Shahidi *et al.* 2009). However, no investigations have been published on the compound N-(1-phenylethyl)formamide in beef or bovine gelatin. In the mixed gelatin class, the volatile compounds with the highest VIP values were ethanol, 2-n-butyl furan, pentadecane, 7-methyl-, 5,5-dimethyl-1,3-dioxan-2-one, and docosanoic acid. The results showed that marker compounds in the mixed gelatin class were positively correlated, nearly identical to the marker compounds in the bovine and porcine gelatin classes.

5 Conclusion

In conclusion, this study successfully investigated profiles of volatile compounds in gelatin samples (porcine, bovine, and mixed) using SPME-GC-MS. The use of multivariate analysis methods specifically PCA and PLS-DA, facilitated the effective identification of porcine, bovine, and mixed gelatin samples. The results showed that marker compounds for porcine gelatin included 2-n-butyl furan, 3-tetradecyn-1-ol, cyclohexane, 1-butenylidene-, trans-2-undecen-1-ol, and 5,5-dimethyl-1,3-dioxan-2-one. In comparison, standard bovine gelatin was distinguished by compounds such as propan-1-one 3-nitro-1-phenyl, pentadecane 7-methyl, cycloundecane 1,1,2-trimethyl, 1-iodo-2-methylundecane, and phthalic acid butyl tridec-2-yn-1-yl ester. Commercial bovine gelatin markers included 1,2-oxathiane, 6-dodecyl-, 2,2-dioxide, sulfurous acid, 2-ethylhexyl hexyl ester, hexadecane, 1-iodo-, docosanoic acid, and hexadecane. Mixed gelatin could be identified by compounds such as ethanol, 2-n-butyl furan, pentadecane, 7-methyl-, 5,5-dimethyl-1,3-dioxan-2-one, and docosanoic acid.

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Conflict of Interest

The authors declare no conflict of interest.

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