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Sensomics combined with Chemometrics approaches of enzymatically hydrolyzed animal by-product proteins using biomimetic sensory-based machine perception techniques and gas chromatography-Olfactometry-mass spectrometry (GC-O-MS)

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ABSTRACT

In this study, pig blood (PB), milk whey (MW), cow gelatin (CG), and pig gelatin (PG) were used to investigate the flavor profiles of hydrolyzed animal byproduct proteins using biomimetic sensory-based machine perception techniques and gas chromatography-olfactometry-mass spectrometry (GC-O-MS). In addition, this study aimed to explore their potential application in the food industry as a food source. In taste patterns and volatile compounds, PB hydrolyzed with pepsin exhibited the most pronounced umami taste and a glassy odor similar to green and almond. However, PB hydrolyzed with alcalase did not exhibit this distinctive characteristic. In addition, the chemometrics approaches of flavor compounds showed that taste patterns and volatile compounds were influenced by the types of substrate, regardless of the utilization of enzyme types. This study provides flavor profiles of hydrolysis by-product proteins (prototypes) that can be used as a basic database for sensomics combined with chemometrics.

1. Introduction

Over the past two decades, the demand for meat and meat products has increased globally, leading to higher livestock production (Sans & Combris, 2015), The amount of by-products generated during the production of edible meat has increased (Seong et al., 2014). In South Korea, approximately one million pigs are slaughtered annually; however, the utilization of byproducts is significantly lower than the total amount produced (Seong et al., 2014). This underutilization has resulted in environmental issues, as well as costs associated with the management, storage, and disposal of these by-products (Waraczewski et al., 2022).

To address these challenges, animal byproducts, particularly those with high protein content, such as blood, whey, and gelatin, have been explored as potential food sources (Chiroque et al., 2023; Smithers, 2008; Waraczewski et al., 2022). Pig blood (PB), often regarded as a waste product from slaughtering, contains 15–17 % hemoglobin and is rich in iron and various minerals (Penteado et al., 1979; Salvador et al., 2010; Toldrá et al., 2016). Consequently, PB has been used as a food source in several countries (Chiroque et al., 2023). Whey, a well-known byproduct of cheese and casein production in the dairy industry, has gained recognition as a valuable source of functional proteins, peptides, lipids, vitamins, and minerals. Similar to milk production, whey production is increasing globally, making it an essential resource for the food, agriculture, and medical markets (Smithers, 2008). Gelatin, which is primarily derived from the hydrolysis of pig and cow cartilage, bones, and collagen, is a protein-based hydrocolloid with unique structural stability and nutritional properties (Waraczewski et al., 2022). It is widely used in the food industry as a gelling, foaming, and texture enhancer (Uddin et al., 2021).

Owing to the potential of animal byproduct proteins (blood, whey, and gelatin) as food sources, their flavors can be enhanced through

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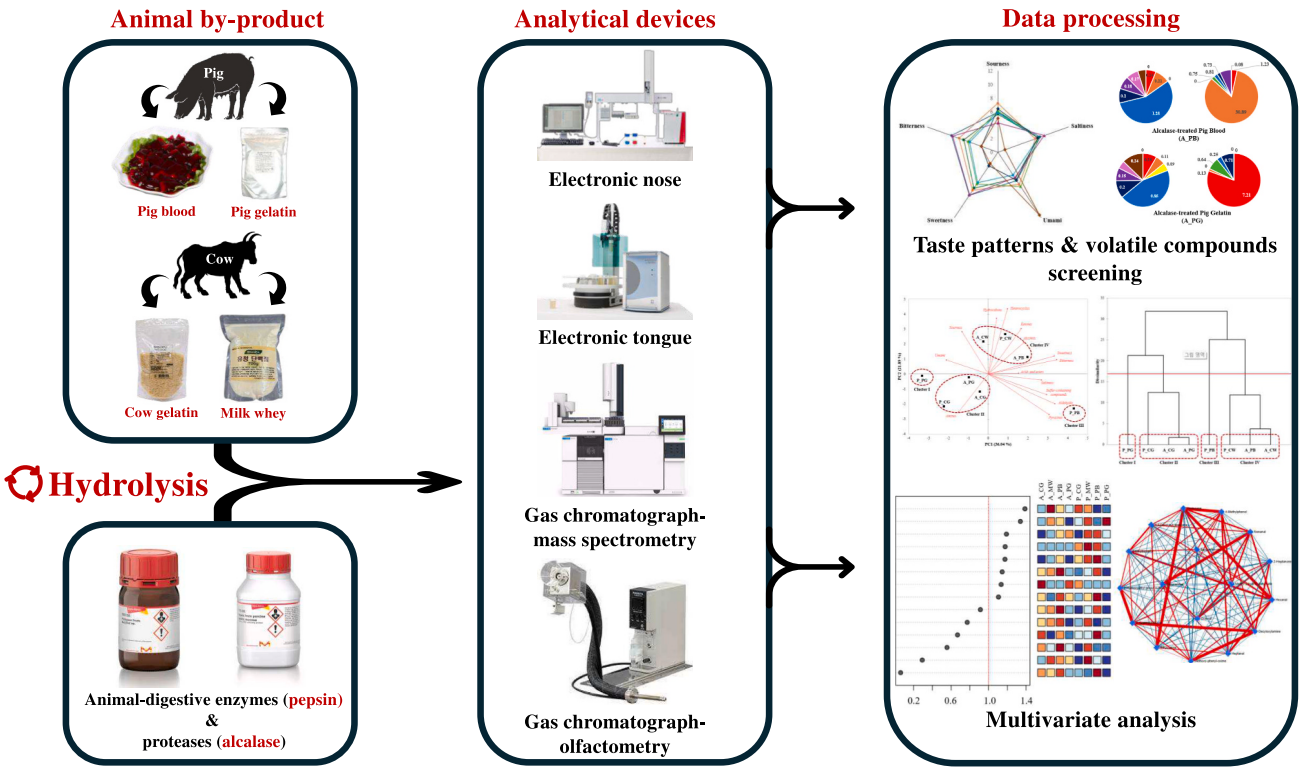


Fig. 1. The flowchart of the experimental procedure is illustrated in Fig. 1.

enzymatic hydrolysis (Zhang et al., 2022). Muradova et al. (2023) confirmed that non-volatile glycosides in food can be converted into volatile compounds through enzymatic hydrolysis, resulting in the production of odor-active substances. Zhang et al. (2022) reported that enzymatic hydrolysis increases the concentration of low-molecular-weight polypeptides, which contribute to enhanced food flavor. Although extensive research on the flavor of meat has been performed, studies focusing on the flavor of meat by-products remain limited. Therefore, this study aimed to analyze the flavor profiles of by-product proteins (blood, whey, and gelatin) derived from slaughtered pigs and cows as food sources. These by-product proteins were hydrolyzed using two commercially available enzymes (alcalase and pepsin), and their flavors were analyzed via sensomics and chemometric approaches. Therefore, this study aimed to investigate the flavor of hydrolyzed by-product proteins (prototypes) via the sensomics (biomimetic sensory-based machine perception techniques; *E*-tongue combines E-nose and gas chromatography-olfactometry-mass spectrometry; GC-O-MS) combined with chemometrics approaches.

2. Materials and methods

2.1. Experimental materials

The PB used in this study was obtained from a local domestic slaughterhouse (Gimhae, Republic of Korea). Milk whey (MW), cow gelatin (CG), and pig gelatin (PG) were purchased from a grocery market (Seoul, Republic of Korea). MW, CG, and PG used in this study were all commercially available in powdered form without any additives. All raw materials were stored at −18 °C until use in the experiment. Animal byproduct proteins were analyzed using hydrolysis with alcalase and pepsin. All enzymes were purchased from Sigma-Aldrich (Steinheim, Germany). The alcalase-treated animal by-product proteins were A_PB: alcalase-treated pig blood; A_MW: alcalase-treated milk whey; A_CG: alcalase-treated cow gelatin; A_PG: alcalase-treated pig gelatin. The pepsin-treated animal by-product proteins were P_PB: pepsin-treated pig blood; P_MW: pepsin-treated milk whey; P_CG: pepsin-treated cow gelatin; P_PG: pepsin-treated pig gelatin. All samples were analyzed.

2.2. Hydrolysis of animal by-product proteins using alcalase and pepsin

Hydrolysis treatments using alcalase and pepsin were performed by adding 50 g of the by-product protein to 1000 mL of distilled water, and the pH was calibrated to 8.00 and 2.00 with 0.1 M NaOH and 0.1 N HCl, respectively, followed by homogenization at 11,000 rpm for 2 min using a homogenizer (HG-15 A, Daihan Scientific Co.). After homogenization, 10 mL of alcalase and pepsin were blended with 1000 mL of distilled water and the substrate mixture, respectively. The hydrolysis was performed in a water bath maintained at 50 °C and 37 °C for 2 h with stirring using a propeller stirrer, respectively. After hydrolysis, the hydrolysate was stirred with a propeller for 20 min in a bath set at 80 °C to terminate the enzymatic activity of the hydrolysate. The inactivated hydrolysate was cooled to room temperature (20–25 °C) for 30 min. The cooled hydrolysate was collected into 1 L bottles using a sieve (35 mesh), funneled, and stored in a refrigerator.

2.3. Taste patterns analysis using electronic (E-tongue)

The taste patterns of enzymatically hydrolyzed animal byproduct proteins were analyzed using an *E*-tongue (ASTREE II, Alpha MOS, Toulouse, France). The E-tongue system consists of five electronic sensors and two indicator sensors related to the basic human perceptible tastes of sourness (AHS), saltiness (CTS), umami (NMS), sweetness (ANS), and bitterness (SCS). The samples were analyzed by diluting 10 mL of each sample with 90 mL of purified water in an *E*-tongue analysis vial. Therefore, taste patterns were analyzed at a volume of 100 mL for each sample. The samples were loaded into the sampler of the *E*-tongue system, and the sensors were immersed in the sample solution for 2 min to measure the respective taste intensities. To prevent contamination from contact between samples during the analysis process, each electronic sensor was rinsed in purified water after analysis. The analysis

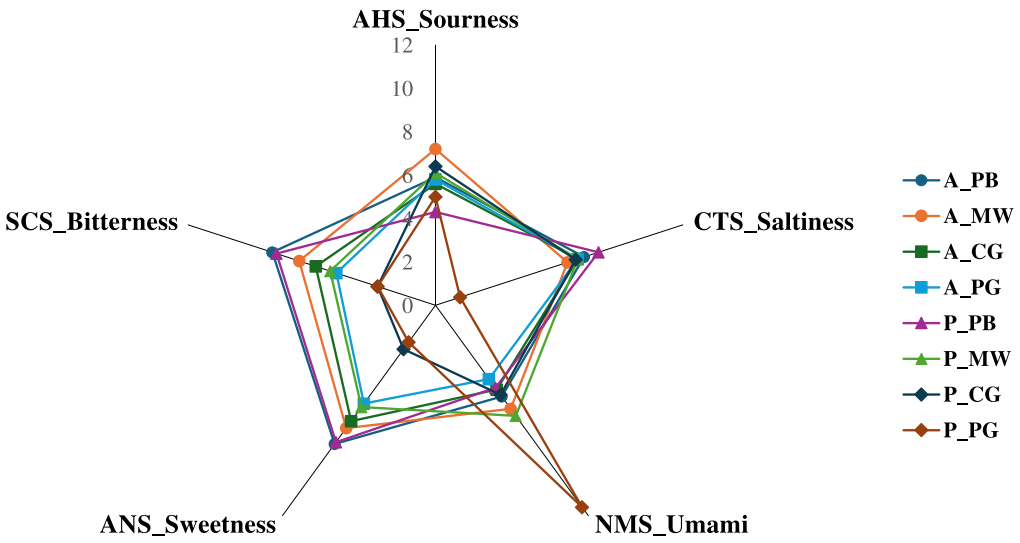


Fig. 2. Taste intensities of hydrolyzed animal by-product proteins with alcalase and pepsin as obtained using the *E*-tongue. A_PB, alcalase-treated pig blood; A_MW, alcalase-treated milk whey; A_CG, alcalase-treated cow gelatin; A_PG, alcalase-treated pig gelatin; P_PB, pepsin-treated pig blood; P_MW, pepsin-treated milk whey; P_CG, pepsin-treated cow gelatin; P_PG, pepsin-treated pig gelatin.

was performed with ten replicates per sample (Yoon et al., 2024).

2.4. Volatile compounds analysis using electronic (E-nose)

The volatile compounds of the enzymatically hydrolyzed animal by-product proteins were analyzed using an *E*-nose (HERACLES Neo, Alpha MOS). Here, 3 mL of each sample were placed in a headspace vial for analysis and stirred at 1200 ×*g* for 20 min at 40 °C to saturate the vial with volatile compounds. The saturated volatile compounds were captured using an autosampler attached to the *E*-nose, and 1000 μL of the captured volatile compounds were injected into the GC injection port equipped on the *E*-nose using a syringe. The analytical conditions were set at a trap absorption temperature of 40 °C, a trap desorption temperature of 250 °C, an acquisition time of 110 s, and a hydrogen gas flow rate of 1 mL/min. The column used for the E-nose analysis was an MXT-5 column (Alpha MOS), and the oven temperature was maintained at 40 °C for 5 s, then ramped up to 270 °C at a rate of 4 °C/s, and held for 30 s. To identify the volatile compounds of the aroma using the E-nose system, the retention index based on carbon atoms was based on Kovat's index library, and the separated peak components were identified using the AcroChemBase (Alpha MOS) of E-nose. All procedures were performed in three replicates per sample (Jeong et al., 2024).

2.5. Volatile compounds analysis using gas chromatography–mass spectrometry (GC–MS) coupled with gas chromatography-olfactometry (GC-O)

Animal byproduct proteins were analyzed using GC–MS (Agilent 7890 A & 5975C, Agilent Technologies) for the quantification of volatile compounds. The headspace method was used to capture the volatile compounds of each sample, using 50/30 μm, divinylbenzene/carboxen/polydimethylsiloxane-coated solid-phase microextraction (SPME, Supelco Inc.). Three milliliters of the sample was placed in a headspace vial, sealed with an aluminum cap, equilibrated at 60 °C for 20 min, and then exposed to SPME fibers for 25 min for capture. After capture, the SPME fibers with adsorbed volatile compounds were analyzed using GC–MS equipped with an HP-5MS column (30 m × 0.25 mm i.d × 0.25 um film thickness, Supelco Inc.) The analytical conditions for GC–MS were an oven temperature of 40 °C followed by an increase to 200 °C at a rate of 5 °C for 5 min, C/min. The injector temperature was set to 220 °C, with a helium flow rate of 1.0 mL/min as the carrier gas (spitless mode). The volatile compounds separated from the total ion chromatogram were classified using a mass spectrum library (NIST 12), and the peak areas of each component were calculated in μg/10^5 g using pentadecane as an internal standard. The identified samples were analyzed using an olfactory detection port (ODP-III, Gerstel Inc.) fitted to the GC–MS to analyze active volatile compounds. The active volatile compounds were determined for 20 min, ranging 5–25 min. The intensity of the perceived active volatile compounds was set as numerical values of 0–4, with higher odor intensity indicated stronger fragrance intensity (Jeong et al., 2024).

2.6. Data processing

The flowchart of the experimental procedure is illustrated in Fig. 1. Among chemometrics, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using XLSTAT software version 9.2 (Addinsoft, New York, NY, USA) to identify patterns in the flavor profile of animal byproduct proteins. Partial least squares-discriminant analysis (PLS-DA) was used to classify the samples using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/) and determine the most important factors contributing to the classification (Yoon et al., 2024). A debiased sparse partial correlation (DSPC) algorithm based on the declassified graphical Least Absolute Shrinkage and Selection Operator (LASSO) modeling procedure was used to identify the correlation pattern of each contributing factor (Marino et al., 2022).

3. Results and discussion

3.1. Taste patterns analysis with E-tongue

This study used the E-tongue, a device that applies biomimetic sensory-based recognition technology using electronic sensors (Zheng et al., 2022). To analyze the taste patterns of enzyme-treated blood, whey, and gelatin from pigs and cows. The results of the taste pattern analysis are shown in Fig. 2. In the samples hydrolyzed with alcalase, the highest AHS value was 7.2 in A_MW, the highest CTS value was 7.2 in A_PB, the highest NMS value was 5.9 in A_MW, the highest ANS value was 7.9 in A_PB, and the highest SCS value was 7.9 in A_PB. In the samples hydrolyzed with pepsin, the highest AHS value was 6.4 in P_CG, the highest CTS value was 7.9 in P_PB, the highest NMS value was 11.5 in P_PG, the highest ANS value was 7.8 in P_PB, and the highest SCS value was 7.7 in P_PB.

PB is associated with bitterness and an astringent taste, as well as

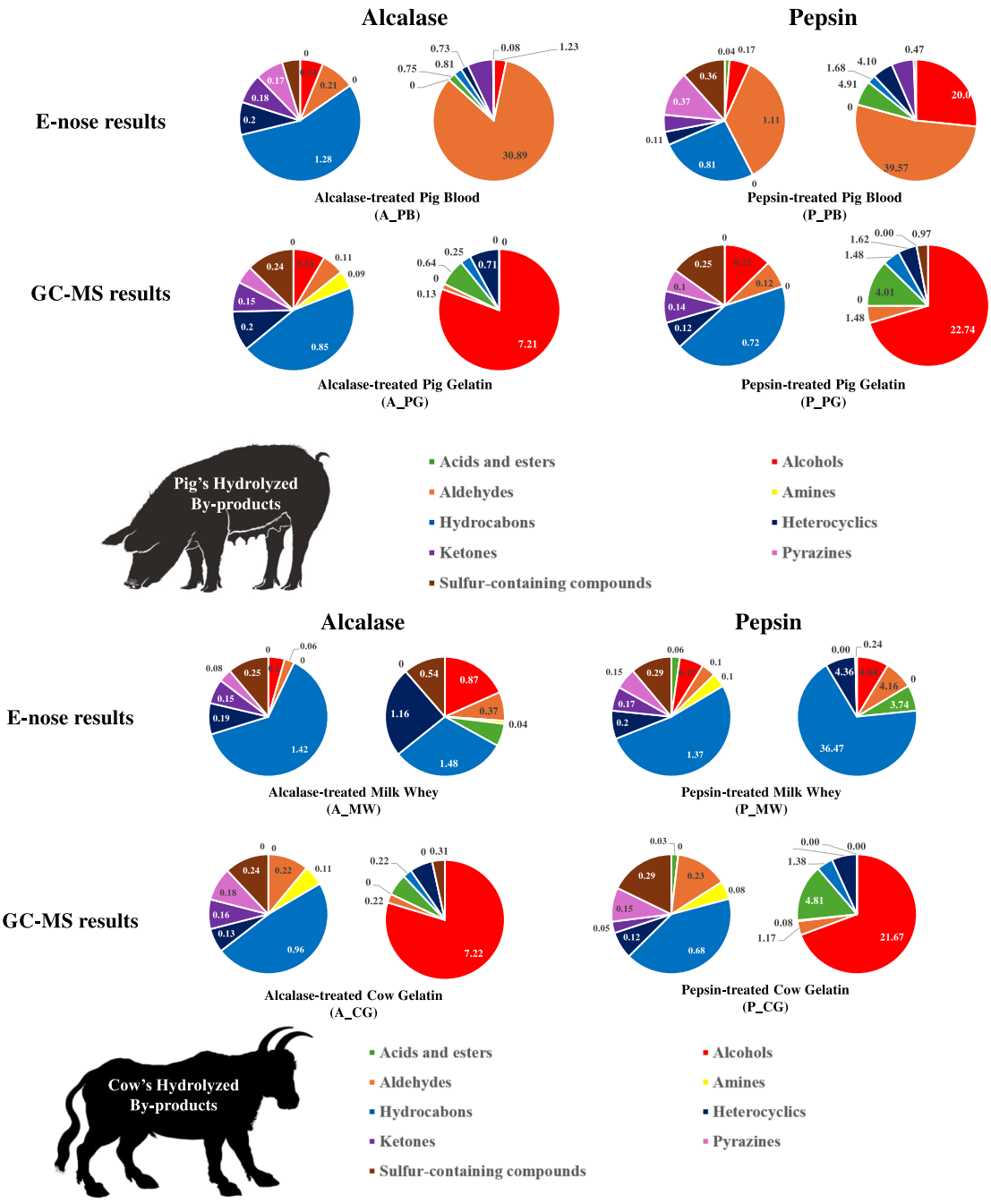


Fig. 3. Composition of hydrolyzed by-product protein volatile compound profiles of cows (A) and pigs (B) using E-nose and GC–MS. A_PB, alcalase-treated pig blood; A_MW, alcalase-treated milk whey; A_CG, alcalase-treated cow gelatin; A_PG, alcalase-treated pig gelatin; P_PB, pepsin-treated pig blood; P_MW, pepsin-treated milk whey; P_CG, pepsin-treated cow gelatin; P_PG, pepsin-treated pig gelatin.

smoky, animal, and fish sauce-like tastes, owing to its hemoglobin content (Li et al., 2020). Aubes-Dafau et al. (1995) reported that hemoglobin hydrolysate hydrolyzed with pepsin exhibited a distinct and strong bitter taste. Furthermore, the major amino acid in pepsin-hydrolyzed PB is leucine, which is associated with bitterness (Fan et al., 2019; Kim, 2022; Kohl et al., 2013). Similarly, the results of this study showed that A_PB and P_PB had higher SCS values than the other samples, with 7.9 and 7.7, respectively). The major sensory attribute of MW hydrolysates is their bitter taste (Leksrisompong et al., 2012). This study analyzed the SCS values of A_MW and P_MW as 6.6 and 5.1, respectively. Although these values were higher than those of the other samples, they were relatively lower than those of A_PB and P_PB. According to Petersen et al. (2005), PG and CG are high in amino acids associated with umami taste (glutamic acid and aspartic acid). In this study, P_PG had a high NMS value (11.5) compared to all the other samples. However, A_PG exhibited the lowest NMS value (4.2). This is likely because pepsin generates more glutamic acid and aspartic acid, the amino acids responsible for PG's flavor of PG, than alcalase (Kirkland et al., 2022). Therefore, pepsin-treated PB has a relatively high umami taste, suggesting that it can enhance the taste of various foods and support rich taste characteristics (McCabe & Rolls, 2007; Wang et al., 2020).

Table 1
Volatile compounds in hydrolyzed animal by-product proteins using electronic nose. (Peak area × 10^3).[1)]

Compounds	RT[1)] (RI[2)])	Sensory description	Alcalase				Pepsin			
			A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG
Acids and esters (3)										
2-Methylpropanoic acid	40.49 (775)	Butter, cheese	ND[3)]	ND	ND	ND	0.04 + 0.04	ND	ND	ND
Methyl pentanoate	46.09 (826)	Apple, sweet	ND	ND	ND	ND	ND	0.06 + 0.01	ND	ND
Propyl decanoate	89.53 (1486)	Fatty	ND	ND	ND	ND	ND	ND	0.03 + 0.03	ND
Alcohol (1)										
2-Methyl-2-propanol	17.53 (489)	Camphor	0.14 + 0.02	0.10 + 0.09	ND	0.16 + 0.04	0.17 + 0.02	0.17 + 0.02	ND	0.13 + 0.01
Aldehydes (10)										
Acetaldehyde	15.22 (438)	Fresh, fruity	0.14 + 0.02	0.06 + 0.01	ND	ND	0.26 + 0.05	0.10 + 0.02	ND	ND
Propenal	16.38 (463)	Almond	ND	ND	ND	0.06 + 0.01	0.06 + 0.00	ND	0.04 + 0.04	0.06 + 0.01
Propanal	17.48 (488)	Nutty	ND	ND	0.15 + 0.09	ND	ND	ND	0.13 + 0.02	ND
3-Methyl butanal	27.29 (652)	Almond	ND	ND	ND	ND	0.18 + 0.02	ND	ND	ND
2-Methyl butanal	28.42 (665)	Almond	0.06 + 0.05	ND	ND	ND	0.11 + 0.01	ND	ND	ND
Hexanal	43.75 (803)	Fresh, grassy	0.11 + 0.03	ND	ND	ND	0.21 + 0.03	ND	ND	ND
Heptanal	54.21 (905)	Grassy	ND	ND	ND	ND	0.05 + 0.04	ND	ND	ND
Benzaldehyde	61.19 (975)	Almond, bitter	ND	ND	ND	ND	0.19 + 0.04	ND	ND	ND
Benzeneacetaldehyde	65.39 (1044)	Grassy, green	0.61 + 0.59	0.52 + 0.58	0.48 + 0.48	0.33 + 0.33	0.32 + 0.28	0.28 + 0.23	0.21 + 0.12	0.25 + 0.18
α-Terpinene-7-al	80.01 (1287)	Fatty	ND	ND	0.07 + 0.01	0.05 + 0.04	0.05 + 0.04	ND	0.06 + 0.05	0.06 + 0.05
Amine (1)										
Trimethylamine	15.19 (437)	Fishy	ND	ND	0.11 + 0.05	0.09 + 0.03	ND	ND	0.08 + 0	ND
Hydrocarbons (2)										
Methylcyclopentane	25.31 (631)		ND	ND	ND	0.09 + 0.02	ND	0.08 + 0.01	ND	ND
Octane	43.39 (800)	Sweet	ND	0.22 + 0.13	ND	ND	ND	0.53 + 0.07	ND	ND
Heterocyclics (3)										
Pyrrole	39.51 (767)	Nutty, cracker	ND	ND	ND	ND	ND	0.04 + 0.03	ND	ND
Caprolactam	80.03 (1288)		ND	ND	ND	ND	ND	0.05 + 0.05	ND	ND
2,4-Dinitrotoluene	91.23 (1524)	Slight	0.20 + 0.04	0.19 + 0.01	0.13 + 0.05	0.11 + 0.10	0.11 + 0.10	0.11 + 0.10	0.12 + 0.10	0.12 + 0.11
Ketones (3)										
Carvone	78.73 (1264)	Basil, bitter	0.07 + 0.00	ND	ND	0.06 + 0.01	0.05 + 0.04	ND	ND	0.05 + 0.05
δ-Octalactone	78.84 (1266)	Coconut	ND	0.06 + 0.01	0.06 + 0.01	ND	ND	0.07 + 0.02	0.05 + 0.04	ND
δ-Nonalactone	85.58 (1400)	Coconut	0.11 + 0.01	0.09 + 0.01	0.10 + 0.02	0.09 + 0.02	0.09 + 0.01	0.10 + 0.01	ND	0.09 + 0.02
Pyrazines (3)										
Trimethylpyrazine	62.39 (1001)	Burnt, bread	ND	ND	ND	ND	0.22 + 0.07	ND	ND	ND
Tetramethylpyrazine	69.21 (1099)	Burnt, nutty	0.08 + 0.02	0.08 + 0.02	0.08 + 0.03	ND	0.06 + 0.01	0.05 + 0.05	0.06 + 0.01	ND
2,3-Diethyl-5-methylpyrazine	72.87 (1160)	Hazelnut	0.09 + 0.02	ND	0.1 + 0.01	0.09 + 0.01	0.09 + 0.00	0.10 + 0.01	0.09 + 0.01	0.10 + 0.02

(*continued on next page*)

Table 1 (*continued*)

Compounds	RT[1)] (RI[2)])	Sensory description	Alcalase				Pepsin			
			A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG
Sulfur-containing compounds (3)										
Carbon disulfide	20.29 (550)	Burnt	0.09 + 0.01	ND	ND	ND	0.15 + 0.01	0.10 + 0.01	ND	ND
2-Methyl-2-propanethiol	22.53 (600)	Sulfurous	0.20 + 0.03	0.14 + 0.13	0.24 + 0.04	0.24 + 0.02	0.21 + 0.02	0.29 + 0.02	0.29 + 0.05	0.25 + 0.01
Furfuryl thioacetate	72.93 (1161)	Burnt, nutty	ND	0.11 + 0.02	ND	ND	ND	ND	ND	ND

[1] RT: retention time. [2)]RI: retention index. [3)]ND: not detected.

3.2. Volatile compounds analysis with E-nose

In this study, we analyzed the volatile compounds in PB, MW, CG, and PG treated with alcalase and pepsin. This was achieved using an E-nose, which is a device that employs biomimetic sensory-based machine perception technology with electronic sensors (Kim et al., 2022). The results are shown in Fig. 3 as the proportion of volatile compounds in the by-products. The peak areas for each volatile compound are listed in Table 1. A total of 29 volatile compounds were identified during the enzymatic hydrolysis of animal byproduct proteins, including three acids and esters, one alcohol, ten aldehydes, one amine, two hydrocarbons, three heterocyclics, three ketones, three pyrazines, and three sulfur-containing compounds. The most volatile compounds identified in the samples hydrolyzed with alcalase were 12 in A_PB, and 10 in A_MW and A_CG. The highest number of volatile compounds identified in the samples hydrolyzed with pepsin was 19 in P_PB, whereas the lowest was 9 in P_PG.

Among the samples, the peak areas of aldehydes were the highest, whereas those of acids and esters were the lowest. The major volatile compounds detected in the by-product proteins treated with alcalase were aldehydes, sulfur-containing compounds, and ketones. The major volatile compounds detected in the by-product proteins treated with pepsin were aldehydes, sulfur-containing compounds, and pyrazines. Aldehydes are primarily generated through amino acid metabolism or fatty acid oxidation (Lomelí-Martín et al., 2021). They are important volatile compounds in foods and flavorings because of their low odor thresholds (Lomelí-Martín et al., 2021). Hexanal is known to have a distinctive aroma profile with a strong fatty green odor at a low threshold (Park, et al., 2024) and was detected as a low peak area in A_PB and P_PB. The content was higher in byproducts treated with pepsin. Benzeneacetaldehyde is a Strecker degradation product of phenylalanine that exhibits a green aroma (Yoon et al., 2024). A large peak area was detected for all samples. The content was higher in the by-products treated with alcalase. Park, et al. (2024) reported 3-methyl butanal and 2-methyl are the major volatile compounds in pork, which were also detected in low amounts in this study. 3-methyl butanal was detected only in P_PB, while 2-methyl butanal was detected only in A_PB and P_PB. The content of 2-methyl butanal was higher in the byproducts treated with pepsin.

Park, Shin, and Choi (2024) reported that volatile compounds in beef are predominantly associated with grassy odors, whereas those in pork are predominantly associated with fruity and sweet odors. However, in this study, benzeneacetaldehyde, which is associated with a grassy aroma, was detected in all samples, whereas hexanal was only detected in samples A_PB and P_PB. Furthermore, the volatile compounds associated with the grassy odor in byproducts A_MW, P_MW, A_CG, and P_CG were lower than those in the pig by-products A_PB and P_PB. This suggests that cow by-products are more sensitive to enzymatic treatment than pig by-products. Notably, different parts of the same animal can contain different volatile compound compositions (Park et al., 2024b).

3.3. Analysis of volatile compounds using GC–MS combined GC-O

The results of the GC–MS analysis of the volatile compounds in eight samples of enzymatically hydrolyzed by-product proteins are shown in Fig. 3, based on the proportion of volatile compounds. The peak areas for each volatile compound are listed in Table 2. A total of 51 volatile compounds were analyzed: nine alcohols, six aldehydes, one amine, one ester, 22 hydrocarbons, four heterocyclics, six ketones, and two sulfur-containing compounds. The most volatile compounds identified in the samples hydrolyzed with alcalase were 21 in A_PB, while the lowest was 8 in A_MW. The highest number of volatile compounds identified in the samples hydrolyzed with pepsin was 29 in P_MW, whereas the lowest was 13 in P_PG. The GC–MS results identified aldehydes as the major volatile compounds of the by-product protein, and a relatively high content of volatile compounds was detected in PB, confirming the differences between the samples. However, unlike the *E*-nose results, acetaldehydes, propenal, propanal, 3-methyl butanal, 2-methyl butanal, benzeneacetaldehyde, and α-terpinene-7-al were not detected among the volatile compounds of the samples, and nonanal, 4-propylbenzaldehyde, and dodecanal were detected in addition.

Park et al. (2024a) reported that aldehydes, ketones, and alcohols were the major volatile compounds in animal proteins. Aldehydes and alcohols were the most abundant volatile compounds identified. Hexanal, also detected in the E-nose, is known to have a green aroma (Park et al., 2024a) and was detected in A_PB and P_PB. Furthermore, benzaldehyde, which was also detected in the E-nose, was reported to have an almond aroma (Yoon et al., 2024) and was detected in all samples. In particular, A_PB and P_PB showed relatively high concentrations. Heptanal, which has a grassy aroma in beef (Dimitrios et al., 2019; Park et al., 2024b), was detected only in P_MW. Heptanal is a characteristic volatile compound that is detected only in cows (Park et al., 2024b). Alcohols, which generally do not contribute significantly to odor compared to aldehydes and ketones (Jeong et al., 2024), were the most abundant volatile compounds in the samples hydrolyzed with pepsin. They were also detected at higher concentrations in P_PB, P_CG, and P_PG than in other samples. Notably, 2-ethylhexanol, which imparts a fruity aroma, was detected in all samples (Liu et al., 2022), and its content was higher in the pepsin-treated samples. Ketones are important volatile compounds, which are representative metabolites of the rumen fermentation process in pork and beef. However, ketones were detected only in samples A_PB and P_PB.

Among the byproduct proteins treated with alcalase and pepsin, more volatile compounds were detected in the samples hydrolyzed with pepsin. Among these, aldehydes and ketones with grassy, fruity, and sweet aromas were particularly higher in samples A_PB and P_PB than in the other samples (park et al., 2024b). Therefore, hydrolyzed PB contains volatile compounds that can be recognized by their odor, which is a characteristic of samples using PB as a substrate.

The odor-active compounds and odor intensities of the hydrolyzed by-product proteins analyzed by GC–MS are shown in Fig. 4. The odor intensity of each odor-active compound was represented on a 0–2 scoring scale. Seventeen odor-active compounds were detected in the salmon byproduct samples, including three alcohols, four aldehydes,

Table 2
Volatile compounds in hydrolyzed animal by-product proteins using GC/MS.[1)]

Volatile compounds	RT[1)]	RI[2)]	Mean ± SD (μg/10^5 g)								I.D.[3)]
			Alcalase				Pepsin				
	(min)		A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG	
Alcohols (9)											
Octenol	13.76	981.73	ND[4)]	ND	ND	ND	0.83 ± 1.18	ND	ND	ND	MS/RI[5)]
2-Ethylhexanol	15.32	1030.19	0.41 ± 0.58	0.40 ± 0.03	0.82 ± 0.04	0.32 ± 0.46	1.92 ± 0.11	1.77 ± 0.04	2.10 ± 0.18	1.79 ± 0.16	MS/RI
2-Butyloctanol	16.24	1059.81	ND	ND	ND	ND	0.26 ± 0.37	ND	ND	ND	MS
1-Octanol	16.61	1071.29	0.31 ± 0.43	ND	ND	ND	1.05 ± 0.05	ND	ND	ND	MS
Tetradecanol	16.62	1071.44	0.06 ± 0.08	ND	ND	ND	ND	0.21 ± 0.10	ND	ND	MS
4-Methylphenol	16.74	1075.09	0.45 ± 0.03	ND	ND	ND	0.67 ± 0.05	ND	ND	ND	MS
1-Hexadecanol	27.55	1469.30	ND	ND	ND	ND	ND	0.20 ± 0.29	ND	ND	MS
Dodecanol	27.56	1469.50	ND	ND	ND	ND	ND	ND	0.11 ± 0.01	0.06 ± 0.08	MS
2,4-Ditert-butylphenol	28.53	1508.54	ND	0.47 ± 0.12	6.40 ± 0.04	6.89 ± 0.58	15.27 ± 0.99	2.46 ± 0.26	19.46 ± 0.10	20.89 ± 0.78	MS
Aldehydes (6)											
Hexanal	7.83	804.04	6.21 ± 0.42	ND	ND	ND	7.84 ± 0.30	ND	ND	ND	MS/RI
Heptanal	11.15	901.84	ND	ND	ND	ND	ND	0.41 ± 0.58	ND	ND	MS/RI
Benzaldehyde	13.12	963.76	23.48 ± 1.67	0.18 ± 0.26	0.08 ± 0.02	0.13 ± 0.03	30.20 ± 1.47	2.07 ± 0.26	0.60 ± 0.10	0.59 ± 0.03	MS/RI
Nonanal	17.64	1102.39	0.89 ± 0.01	0.16 ± 0.23	0.14 ± 0.00	ND	0.45 ± 0.02	0.69 ± 0.07	0.13 ± 0.00	ND	MS/RI
4-Propylbenzaldehyde	22.50	1268.10	0.28 ± 0.02	ND	ND	ND	1.08 ± 0.05	0.78 ± 0.07	0.44 ± 0.62	0.83 ± 0.07	MS
Dodecanal	25.96	1403.98	0.03 ± 0.04	0.03 ± 0.04	ND	ND	ND	0.21 ± 0.02	ND	0.06 ± 0.08	MS
Amine (1)											
Decyloxyamine	16.23	1059.53	ND	0.04 ± 0.06	ND	ND	ND	ND	0.08 ± 0.12	ND	MS
Ester (1)											
Methyl 2-hydroxybenzoate	20.35	1194.69	0.75 ± 1.06	0.29 ± 0.08	0.53 ± 0.04	0.64 ± 0.12	4.91 ± 0.27	3.74 ± 0.48	4.81 ± 0.21	4.01 ± 0.20	MS

Volatile compounds	RT[1)]	RI[2)]	Mean ± SD(μg/10^5 g)								I.D.[3)]
			Alcalase				Pepsin				
	(min)		A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG	
Hydrocarbons (22)											
2-Methylheptane	6.71	<800	ND	ND	ND	ND	ND	0.55 ± 0.43	ND	ND	MS
1-Methyl-3-ethylcyclopentane	7.44	<800	ND	ND	ND	ND	ND	0.31 ± 0.11	ND	ND	MS
Nonane	7.76	801.60	ND	0.34 ± 0.48	ND	ND	ND	0.25 ± 0.35	ND	ND	MS
Octane	7.78	802.35	ND	0.35 ± 0.49	ND	ND	ND	16.08 ± 1.18	ND	ND	MS/RI
4-Methyloctane	9.88	868.06	ND	ND	ND	ND	ND	1.33 ± 0.19	ND	ND	MS
1-Hexyl-3-methylcyclopentane	13.98	987.86	ND	ND	ND	ND	ND	1.70 ± 0.37	ND	ND	MS
Eicosane	14.85	1014.26	ND	ND	ND	ND	ND	0.62 ± 0.87	ND	ND	MS
2,5-Dimethylnonane	15.04	1020.89	ND	ND	ND	ND	ND	0.27 ± 0.38	ND	ND	MS
4-Methyldecane	15.12	1023.35	ND	ND	ND	ND	ND	1.64 ± 0.38	ND	ND	MS
Dodecane	16.22	1059.09	ND	ND	ND	ND	ND	4.56 ± 1.87	ND	ND	MS
2,6,10-Trimethyltetradecane	16.25	1059.97	ND	ND	ND	ND	0.26 ± 0.37	ND	ND	ND	MS

(*continued on next page*)

Table 2 (*continued*)

Volatile compounds	RT[1)]	RI[2)]	Mean ± SD(μg/10^5 g)								I.D.[3)]
			Alcalase				Pepsin				
	(min)		A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG	
2,6,7-Trimethyldecane	16.30	1061.75	ND	ND	ND	ND	ND	0.24 ± 0.33	ND	ND	MS
Tridecane	16.39	1064.45	ND	ND	ND	ND	ND	0.27 ± 0.38	ND	ND	MS
Cyclooctane	16.60	1070.98	0.35 ± 0.49	ND	ND	ND	ND	ND	ND	ND	MS
Ethylxylene	17.09	1085.58	ND	ND	ND	0.053 ± 0.07	ND	ND	ND	ND	MS
4-Isopropyltoluene	17.10	1085.97	ND	ND	ND	0.04 ± 0.06	ND	ND	0.09 ± 0.13	0.05 ± 0.07	MS
Ethyldimethylbenzene	17.11	1086.29	ND	ND	ND	ND	ND	0.17 ± 0.24	ND	ND	MS
Undecane	17.50	1097.60	ND	0.25 ± 0.01	0.22 ± 0.05	ND	0.24 ± 0.34	0.55 ± 0.11	0.17 ± 0.23	0.44 ± 0.01	MS/RI
3,3-Dimethylhexane	17.58	1099.91	ND	ND	ND	ND	ND	0.33 ± 0.47	ND	ND	MS
1,2,4,5-Tetramethylbenzene	18.02	1116.16	ND	ND	ND	0.04 ± 0.05	ND	0.25 ± 0.02	0.05 ± 0.07	ND	MS
1,3-Ditert-butylbenzene	21.98	1254.63	0.46 ± 0.65	0.51 ± 0.04	ND	0.12 ± 0.18	1.18 ± 0.00	7.35 ± 0.20	1.00 ± 0.01	0.99 ± 0.06	MS
Hexadecane	22.62	1273.13	ND	0.03 ± 0.04	ND	ND	ND	ND	0.07 ± 0.09	ND	MS

Volatile compounds	RT[1)]	RI[2)]	Mean ± SD(μg/10^5 g)								I.D.[3)]
			Alcalase				Pepsin				
	(min)		A_PB	A_MW	A_CG	A_PG	P_PB	P_MW	P_CG	P_PG	
Heterocyclics (4)											
Methoxy-phenyl-oxime	11.27	906.14	ND	1.11 ± 0.10	0.56 ± 0.06	0.65 ± 0.15	3.33 ± 0.56	4.36 ± 0.71	2.01 ± 0.15	0.94 ± 1.32	MS
Ethylmethylmaleimide	21.32	1230.53	0.13 ± 0.19	ND	ND	ND	0.77 ± 0.16	ND	ND	ND	MS
Indole	23.06	1291.46	0.46 ± 0.65	ND	ND	ND	ND	ND	ND	ND	MS/RI
6-Aza-5,7,12,14-tetrathiapentacene	25.31	1379.38	0.14 ± 0.01	0.05 ± 0.07	ND	0.06 ± 0.09	ND	ND	0.12 ± 0.17	0.68 ± 0.17	MS
Ketones (6)											
2-Heptanone	10.83	893.41	0.76 ± 0.08	ND	ND	ND	0.10 ± 0.84	ND	ND	ND	MS
2-Octanone	14.14	992.15	ND	ND	ND	ND	1.09 ± 0.02	ND	ND	ND	MS
Acetophenone	16.54	1069.08	0.22 ± 0.31	ND	ND	ND	0.73 ± 0.03	ND	ND	ND	MS
2-Nonanone	17.29	1091.52	0.46 ± 0.03	ND	ND	ND	0.78 ± 0.01	ND	ND	ND	MS
2-Decanone	20.23	1190.74	0.27 ± 0.02	ND	ND	ND	0.30 ± 0.00	ND	ND	ND	MS
3-Butylcyclohexenone	22.34	1261.08	0.82 ± 0.04	ND	ND	ND	1.43 ± 0.04	ND	ND	ND	MS
Sulfur containing compounds (2)											
2-Hydroxyethyl propyl sulfide	20.70	1206.82	0.08 ± 0.11	ND	ND	ND	0.47 ± 0.67	0.24 ± 0.34	ND	0.97 ± 0.05	MS
Cystine	32.04	1659.91	ND	0.54 ± 0.76	0.31 ± 0.43	ND	ND	ND	ND	ND	MS

[1] RT: retention time. [2)]RI: retention index. [3)]I.D.: identification. [4)]ND: not detected. [5)]MS/RI: identified compounds using both MS and RI.

one amine, one ester, one heterocyclic, four hydrocarbons, two ketones, and one sulfur-containing compound. Among the samples hydrolyzed with alcalase, the most odor-active compounds were A_PB and A_CG, and among the samples hydrolyzed with pepsin, the most odor-active compound was P_PB.

Among the odor-active compounds, hexanal, benzaldehyde, nonanal, and 2-nonanone were recognized as major odor-active compounds. These odor-active compounds have green and almond aromas, and are perceived as good odors. Hexanal, which produces green and fruity odors (Leksrisompong et al., 2010), was perceived in A_PB and P_PB with an odor intensity of 1. Benzaldehyde, which produces almond and fruity odors (Lomelí-Martín et al., 2021), was also perceived in A_PB and P_PB with an odor intensity of 2. Moreover, nonanal, which produces green and fruity odors was perceived in A_CG, P_PB, and P_CG with an odor intensity of 2 (Bayraktar & Onoğur, 2011). The odor-active compounds did not show any differences based on hydrolysis. However, regarding the substrate used, the highest amount of odor-active compounds was detected in hydrolyzed PB. Therefore, the substrate influenced the odor-active compounds more than the enzyme.

There was a difference between the volatile compounds detected

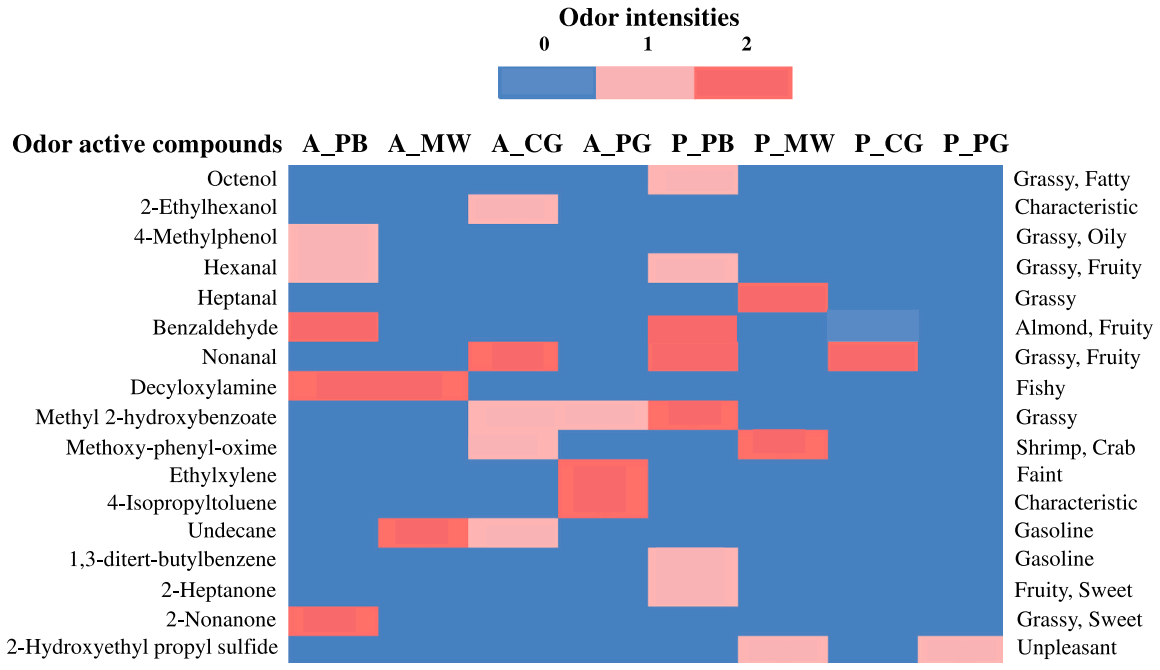


Fig. 4. Odor description and intensity in hydrolyzed animal by-product proteins using GC-O. A_PB, alcalase-treated pig blood; A_MW, alcalase-treated milk whey; A_CG, alcalase-treated cow gelatin; A_PG, alcalase-treated pig gelatin; P_PB, pepsin-treated pig blood; P_MW, pepsin-treated milk whey; P_CG, pepsin-treated cow gelatin; P_PG, pepsin-treated pig gelatin.

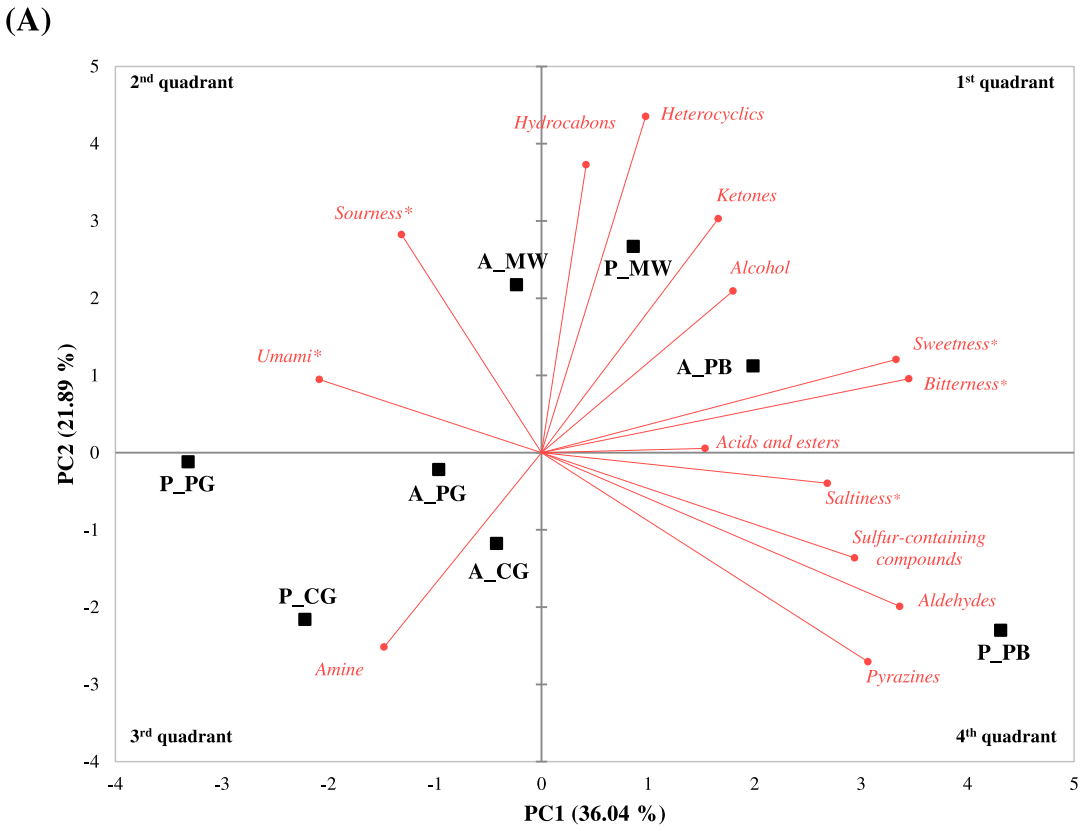
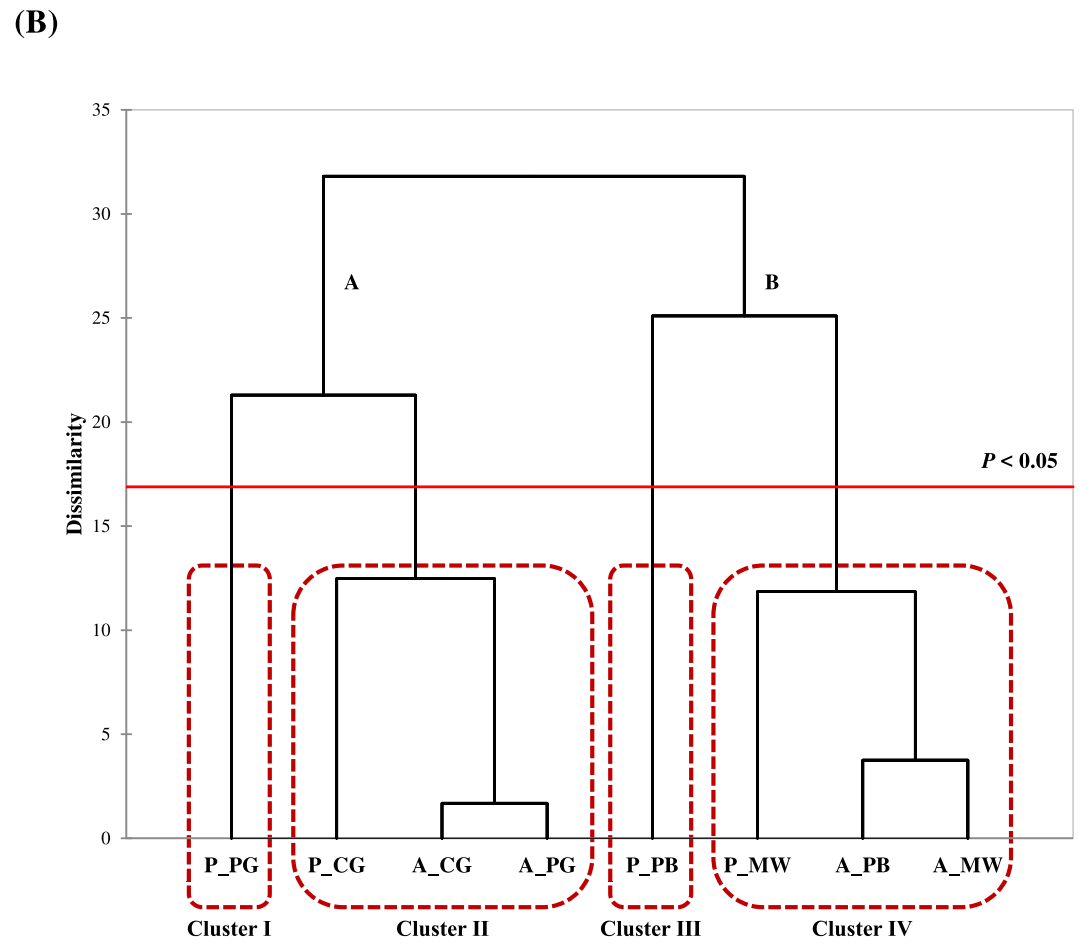


Fig. 5. PCA (A), HCA (B), and PLS-DA (C, D), correlation network (E) result of chemosensory properties in hydrolyzed animal by-product with alcalase and pepsin by E-tongue and E-nose. * indicates each of the taste patterns. A_PB, alcalase-treated pig blood; A_MW, alcalase-treated milk whey; A_CG, alcalase-treated cow gelatin; A_PG, alcalase-treated pig gelatin; P_PB, pepsin-treated pig blood; P_MW, pepsin-treated milk whey; P_CG, pepsin-treated cow gelatin; P_PG, pepsin-treated pig gelatin.



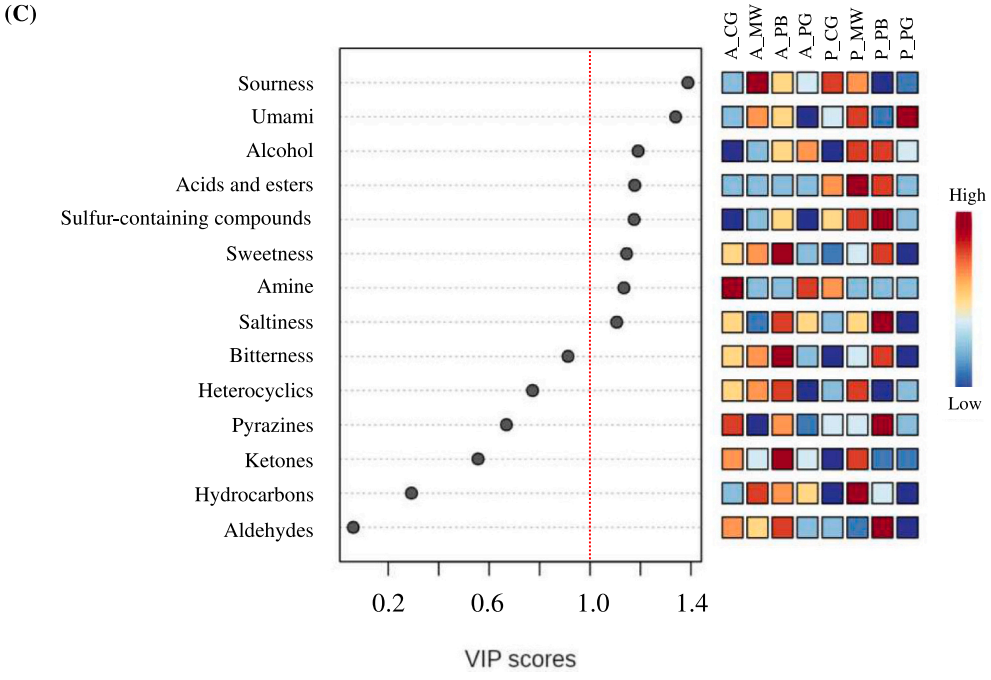


Fig. 5. (*continued*).

using the *E*-nose and GC-O-MS. The difference in the results may be due to the different capture methods and capture temperatures of the two instruments. The volatile compounds of the E-nose were identified using a library built into the software, whereas GC–MS identified the volatile compounds using the fragmentation pattern. In addition, the results of E-nose analysis may differ from those of GC–MS because it is intended to identify patterns in the overall composition rather than individual volatile compounds (Yoon et al., 2023).

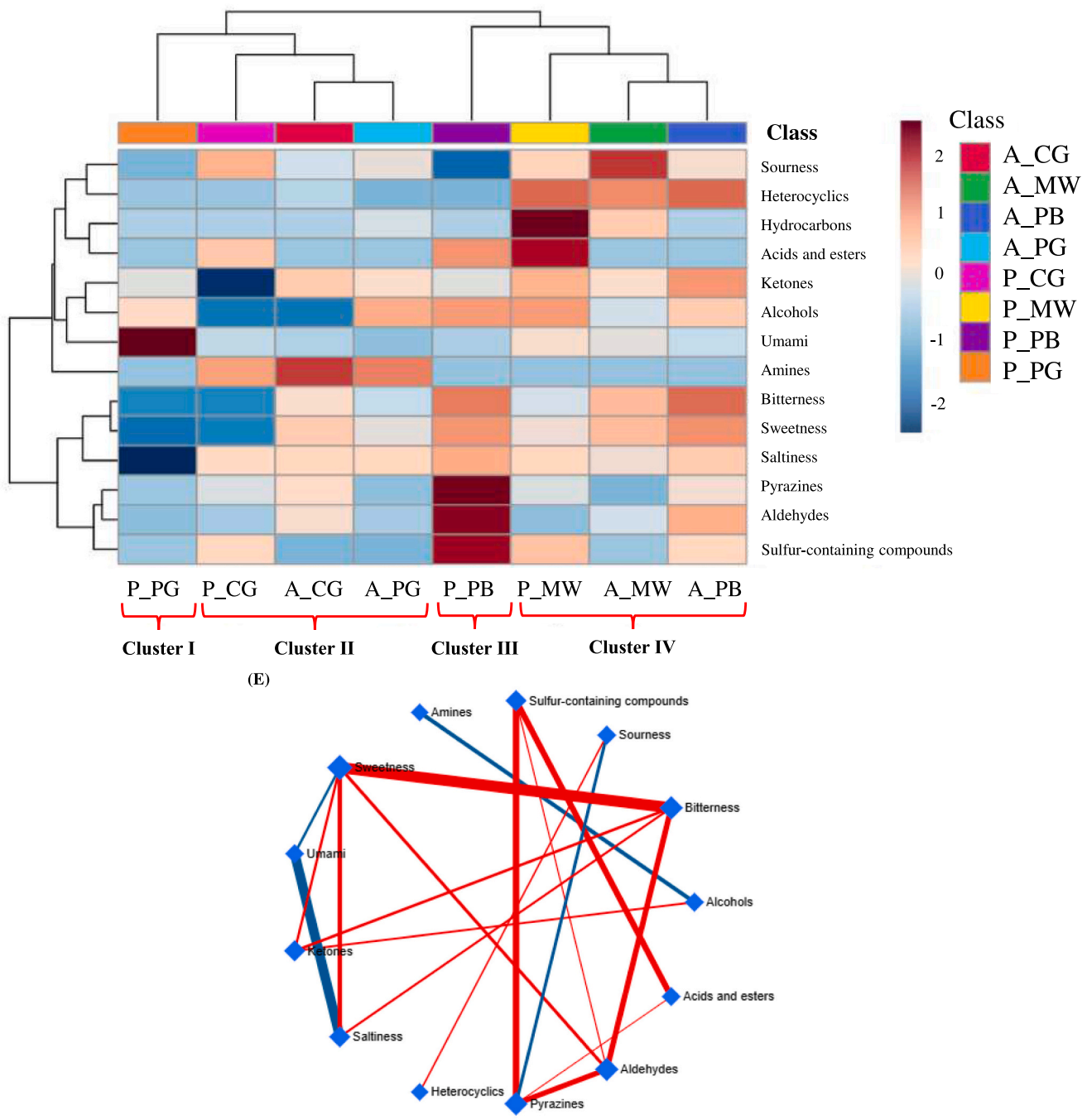


Fig. 5. (*continued*).

3.4. Chemometric approaches results

This study used E-tongue, E-nose, and GC-O-MS to analyze taste patterns, volatile compounds, and odor-active compounds in enzymatically hydrolyzed by-product proteins. PCA and HCA are methods used to analyze the correlations between samples and variables, using various variables to understand the similarities and patterns among samples (Granato et al., 2018). PLS-DA was applied to identify the samples and investigate the most significant contributors (variable importance projection; VIP) to the classification (Hong et al., 2024). Furthermore, the DSPC algorithm was used to represent a correlation network based on the declassified graphical LASSO modeling procedure (Marino et al., 2022). Therefore, PCA, HCA, PLS-DA, and correlation networks were used to identify the patterns, dendrograms, and key factors in the classification of the samples.

3.4.1. Chemometric approaches of taste patterns and volatile compounds

The results of the PCA biplot are shown in Fig. 5A. The variance explained by PC1 was 36.04 %, whereas that explained by PC2 was 21.89 %, resulting in a total variance of 57.93 %. Both P_MW and A_PB

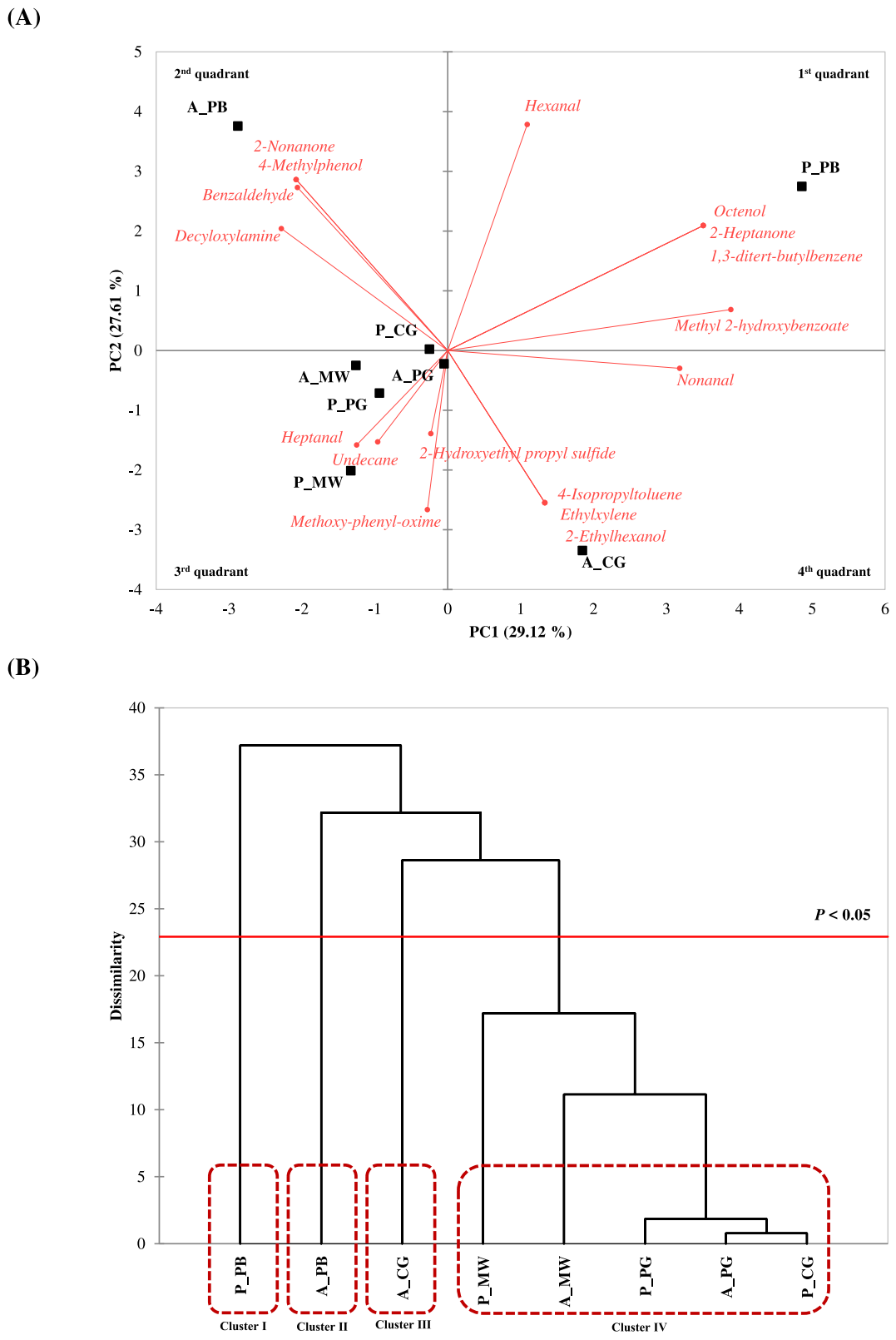
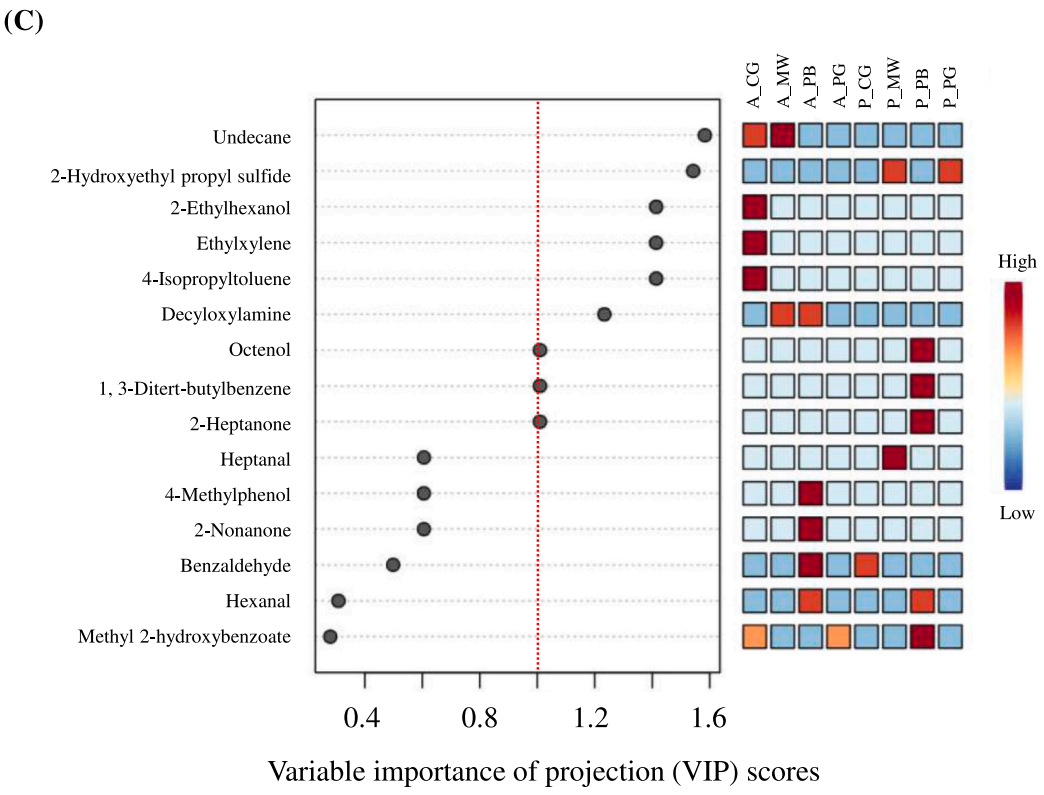


Fig. 6. PCA (A), HCA (B), and PLS-DA (C, D), correlation network (E) result of odor-active compounds in hydrolyzed animal by-product with alcalase and pepsin using GC-O. A_PB, alcalase-treated pig blood; A_MW, alcalase-treated milk whey; A_CG, alcalase-treated cow gelatin; A_PG, alcalase-treated pig gelatin; P_PB, pepsin-treated pig blood; P_MW, pepsin-treated milk whey; P_CG, pepsin-treated cow gelatin; P_PG, pepsin-treated pig gelatin.

are located in the 1st quadrant, indicating positive directions for both PC1 and PC2. This suggests the influence of sweetness, bitterness, hydrocarbons, heterocyclic compounds, acids, esters, ketones, and alcohols. A_MW, observed in the 2nd quadrant, was negatively correlated with PC1 and positively correlated with PC2, indicating the influence of sourness and umami. P_PG, A_PG, P_CG, and A_CG were



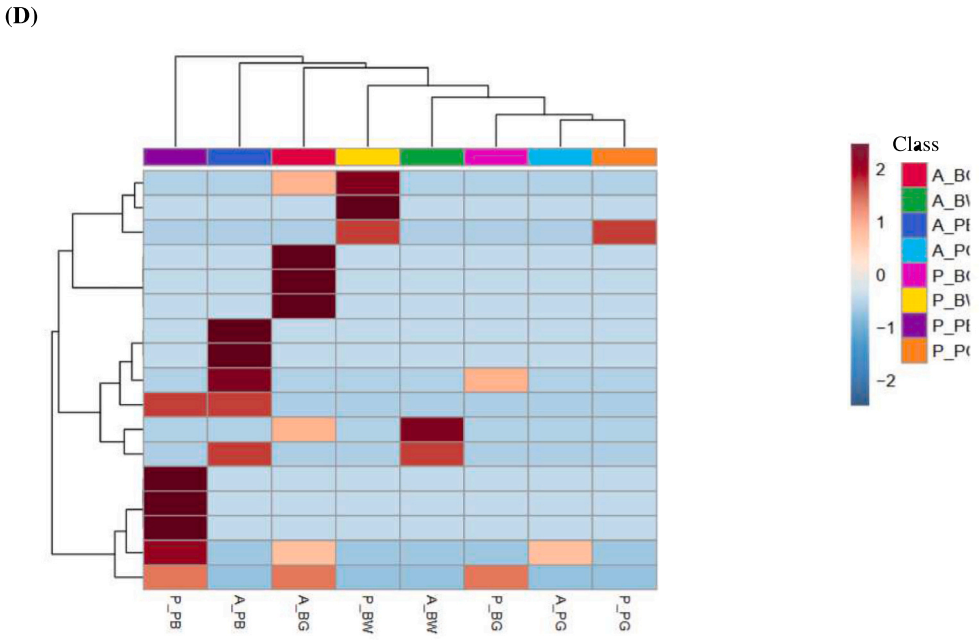


Fig. 6. (*continued*).

positioned in the 3rd quadrant, displaying negative directions for both PC1 and PC2, indicating the influence of amines. Meanwhile, P_PB was situated in the 4th quadrant, with a positive direction for PC1 and a negative direction for PC2, suggesting the influence of saltiness, sulfur-containing compounds, aldehydes, and pyrazines. The HCA results are shown in Fig. 4B, which identified a total of four clusters. Cluster I included P_PG, whereas cluster II included A_PG, A_CG, and P_CG. Cluster III was represented by P_PB and cluster IV included A_PB, A_MW, and P_MW. As shown in Fig. 4B, the samples are classified into clusters A and B, which exhibit the most remarkable dissimilarity, followed by subclusters III and IV. Clusters I and II demonstrated the next highest degrees of dissimilarity. With relatively less dissimilarity, gelatin by-products are classified as cluster A, while other by-products are categorized as cluster B. In cluster A, P_PG was separated because of its umami flavor, which distinguished it from the other samples, whereas in cluster B, P_PB was separated because of its aroma (green and almond), which distinguished it from the other samples. Chemometrics approaches (PCA and HCA) of taste patterns and volatile compounds

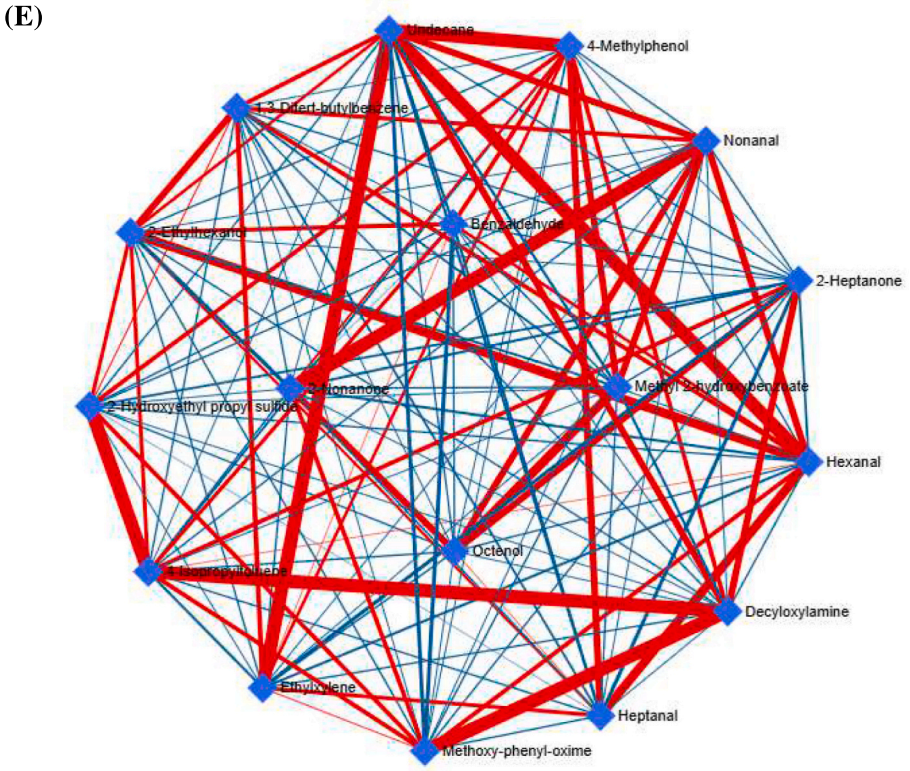


Fig. 6. (*continued*).

resulting from hydrolyzed byproduct proteins showed that classification was primarily based on the substrate type rather than the enzyme used for hydrolysis. In particular, PB was distinctly separated from the other byproducts owing to its high abundance of volatile compounds.

Fig. 4C shows the results of PLS-DA, which is a widely used classification method for pattern identification in data analysis. Fig. 5D shows the results of HCA based on variable importance in projection (VIP) scores. These methods are useful for modeling multiple linear regressions when there are numerous interrelated parameters and observations (Liang et al., 2023). In this study, PLS-DA was employed to analyze eight samples and identify the chemosensory properties that contributed most significantly to their classification. VIP scores were calculated to examine important chemosensory characteristics, with variables having scores greater than one generally considered key contributors to the classification. Higher VIP scores indicated greater differences between clusters and were deemed more important for classification (Yoon et al., 2024). The classification of the eight samples is shown in Fig. 4C, where the most contributing variables (VIP scores $\geq$1) identified in taste patterns include sourness, umami, sweetness, and saltiness. The significant volatile compounds identified include alcohols, acids, esters, sulfur-containing compounds, and amines. Moreover, the clusters shown in Fig. 5D are consistent with those shown in Fig. 4B, indicating that the samples within each cluster are identical.

The correlation network of the taste patterns associated with the volatile compounds detected by the electronic sensor is shown in Fig. 5E. This network, created using the DSPC algorithm, visually represents the connections between various metabolites derived from samples (Marino et al., 2022). The network consists of nine volatile compounds and five taste patterns, forming 14 nodes. Each node corresponds to a metabolite and the edges represent the correlations between them. The correlation network provides a comprehensive overview, highlighting positive correlations (red lines) and negative correlations (blue lines) among the metabolites (Boban et al., 2024).

3.4.2. *Chemometrics approaches of odor-active compounds*

This study analyzed the odor-active compounds in byproduct proteins using GC-O-MS to identify the relationship between these compounds and the aroma of the samples. The PCA biplot results are shown in Fig. 6A. The variance explained by PC1 was 29.12 % and that explained by PC2 was 27.61 %, yielding a total variance of 56.71 %. Sample P_PB was located in the 1st quadrant, showing the positive influence of hexanal, octenol, 2-heptanone, 1,3-di-tert-butylbenzene, and methyl 2-hydroxybenzoate on both PC1 and PC2. A_PB and P_CG are positioned in the 2nd quadrant, indicating a negative direction for PC1 and a positive direction for PC2. This suggested the influence of 2-nonanone, 4-methylphenol, benzaldehyde, and decyloxylamine. In the 3rd quadrant, A_PG, A_MW, P_PG, and P_MW were negatively positioned in both PC1 and PC2, indicating the influence of heptanal, undecane, 2-hydroxyethyl propyl sulfide, and methoxy-phenyl-oxime. Finally, A_CG was in the 4th quadrant with a positive direction for PC1 and a negative direction for PC2, suggesting an influence of nonanal, 4-isopropyltoluene, ethylxylene, and 2-ethylhexanol. The HCA results are shown in Fig. 6B, which identified a total of four clusters. P_PB was classified as cluster I, A_PB as cluster II, and A_CG as cluster III. In addition, A_MW, A_PG, P_CG, P_MW, and P_PG were grouped into cluster IV. Notably, the PB byproduct proteins were categorized into clusters I and II, whereas the other byproduct proteins were categorized into clusters III and IV. This suggests that PB has characteristics that distinguish it from other byproduct proteins. Similar to the results of the chemometrics approaches of volatile compounds and taste patterns (Fig. 5B), Fig. 6B shows that the classification is based more on the substrate than on the hydrolysis treatment.

The results of PLS-DA, a commonly used method for pattern identification in data analysis, are presented in Fig. 6C (Liang et al., 2023). In Fig. 5D, the HCA results based on VIP scores are shown. The variables with the highest contributions to the classification (VIP scores $\geq$1) included undecane, 2-hydroxyethyl propyl sulfide, 2-ethylhexanol, ethylxylene, 4-isopropyltoluene, decyloxylamine, octenol, 1,3-di-tert-

butylbenzene, and 2-heptanone in the odor-active compounds. The clusters shown in Fig. 6D correspond to those presented in Fig. 6B, with identical samples within each cluster.

Fig. 6E shows the correlation network between the odor-active compounds detected using GC-O-MS. The network comprised 17 nodes representing odor-active compounds with edges indicating correlations among them. This correlation network provides a comprehensive overview illustrating both positive (red lines) and negative (blue lines) relationships among the metabolites (Boban et al., 2024).

4. Conclusion

This study analyzed the flavor profiles of enzymatically hydrolyzed animal proteins, specifically blood, whey, and gelatin, which are often regarded as by-products and typically discarded. *E*-tongue analysis indicated that pepsin-treated PG had a richer umami taste than the other byproduct proteins. In addition, E-nose analysis revealed that both pepsin- and alcalase-treated PB were rich in volatile compounds that produced green and almond aromas. Notably, higher levels of pleasant odors were detected in pepsin-treated PB. The GC-O-MS analysis confirmed that the hydrolyzed PB samples contained abundant odor-active compounds, consistent with the findings of the E-nose analysis. Overall, the experimental results suggest that PB treated with alcalase and pepsin is rich in pleasant, odor-active compounds, whereas pepsin-treated PG exhibits a higher umami intensity than other byproduct proteins. Furthermore, sensomics approaches (taste patterns, volatile compounds, and odor-active compounds) revealed that substrate type had a higher significant influence than the utilization of enzyme types. This study is expected to serve as a foundational database for the potential utilization of enzymatically hydrolyzed animal by-product proteins (prototypes) as ingredients in the food industry.

CRediT authorship contribution statement

Hee Sung Moon: Writing – original draft, Methodology, Conceptualization. **Se Young Yu:** Methodology, Formal analysis. **Younglan Ban:** Methodology, Formal analysis. **Hyeonjin Park:** Methodology, Formal analysis. **Seong Jun Hong:** Methodology. **Kyeong Soo Kim:** Writing – review & editing. **Hyun-Wook Kim:** Writing – review & editing. **Eun Ju Jeong:** Writing – review & editing. **Eui-Cheol Shin:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this paper.

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Data availability

Data will be made available on request.

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