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Characterization of enzyme-hydrolyzed vegetable proteins from mung bean, soybean, and pea and their roles in the formation of volatile compounds by thermal interaction with reducing sugars

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The development of plant-based foods with desirable meaty and savory flavors remains challenging due to differences in protein composition and flavor precursors compared with animal proteins. The objective of this study was to systematically compare the physicochemical properties and flavor-producing potentials of enzyme-hydrolyzed vegetable proteins (EVPs) derived from mung bean, soybean and pea. Degree of hydrolysis, molecular weight distribution, and free amino acid profiles were determined, and volatile compounds generated through Maillard reactions with reducing sugars were analyzed. Our results showed that the mung bean EVP exhibited the highest degree of hydrolysis, whereas that from soybean showed the lowest. Pea EVP contained the highest total content of free amino acids, while that of soybean contained the lowest. Leucine and lysine were abundant in all EVPs, whereas sulfur-containing amino acids were relatively limited. Principal component analysis revealed that both EVP type and reducing sugar significantly influenced volatile compound profiles. EVPs from mung bean and pea produced greater amounts of Strecker aldehydes, heterocyclic compounds, and sulfur-containing compounds than soybean hydrolysate. These findings demonstrate that the intrinsic properties of EVPs critically influence Maillard reaction-driven flavor formation and provide practical insights for selecting plant protein sources to enhance flavor characteristics in plant-based food applications.

There has been a continuous rise in the consumption of plant-based proteins as substitutes for animal-derived proteins, particularly in the development of meat analogs and flavoring ingredients[1]. Among various plant protein sources, soy protein has been most extensively studied and utilized due to its high protein content, wide availability, and favorable functional properties[2]. However, the widespread use of soy protein is associated with several limitations, including allergenicity and characteristic off-flavors, which may restrict its broader application in food formulations[3].

Mung bean and pea proteins have recently attracted increasing attention as alternative legume-derived protein sources owing to their balanced amino acid compositions and growing utilization in plant-based foods[4]. These legumes exhibit compositional similarities to soybean protein and, therefore, represent promising candidates for the production of enzyme-hydrolyzed vegetable proteins (EVPs). Despite this potential, comprehensive studies systematically evaluating EVPs derived from mung bean, soybean, and pea under identical processing conditions remain limited, particularly, with respect to differences in physicochemical properties and Maillard reaction-derived volatile compounds.

One of the major challenges in the application of plant proteins as flavoring agents is their inherent grassy and/or beany flavor. Enzymatic hydrolysis has been widely employed to modify plant proteins, as it enables the controlled generation of free amino acids and short peptides that serve as key precursors for Maillard reactions, while avoiding undesirable by-products associated with chemical hydrolysis[5–9]. The Maillard reaction between reducing sugars and amino compounds plays a crucial role in the formation of aroma-active compounds, including Strecker aldehydes and

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heterocyclic compounds, which are essential for the development of meat-like flavors[10–13]. It has been reported that meat-like aromas could be produced by enzyme-hydrolyzed soy proteins when heated with sugars[14]. A recent study on the Maillard reaction of EVPs from mung beans and peas showed a wide range of volatile compounds that played critical roles in flavor development; these included thiophenes, such as 3-methyl-2-thiophene and 2,5-thiophenedicarboxaldehyde, which had been identified as contributors to cooked meat-like flavors[15]. Also, some nitrogen-containing heterocyclic compounds, such as 2,3-dimethylpyrazine and 2-pentylpyridine, were detected along with Strecker aldehydes, indicating their potential contribution to meat-like flavor development through the Maillard reaction[15].

Although previous studies have reported the formation of meat-like aroma compounds from EVPs derived from legume sources, such as soybean, mung bean, or pea[14,15], a comparative evaluation of these legume-derived EVPs for volatile formation, in particular, in relation to their physicochemical properties, has not been reported yet. Therefore, the objective of this study was to comparatively investigate EVPs prepared from mung bean, soybean, and pea by examining their degree of hydrolysis, molecular weight distribution, free amino acid profiles, and volatile compounds generated through thermal reactions with reducing sugars.

Results

Degree of hydrolysis

Figure 1 shows the DH results of the EVPs. The DH of the mung bean, soybean, and pea proteins reached 78.6, 52.1, and 63.3% at 10 h, respectively. In this study, the DH values were slightly higher than those reported in a previous investigation on legumes, which showed DH ranges of 20 to 31% for Alcalase and 18 to 51% for Flavourzyme treatments[16]. This enhancement in DH might be attributed to the sequential action of Flavourzyme, which further hydrolyzes peptides generated by the initial Alcalase treatment, thereby promoting an additional phase of enzymatic breakdown[17]. Utilizing a mixture of enzymes has been reported to enhance the efficiency of protein hydrolysis compared to applying a single enzyme alone[18]. In addition, hydrolysis using an individual enzyme has been reported to result in lower DH values compared with sequential hydrolysis[18]. It was found that the DH was 11.9% when only Flavourzyme was used in chickpea hydrolysis; however, the DH value increased significantly to 50.2% when Alcalase and Flavourzyme were applied simultaneously[19]. Differences in the DH can lead to variations in the reactivities and functional properties of hydrolysates, ultimately contributing to diverse flavor profiles formed during the Maillard reaction.

Hydrolysates' molecular weight distribution

To evaluate the molecular weight profiles of hydrolyzed proteins from mung bean, soybean, and pea, gel permeation chromatography was employed (Table 1). Enzymatic hydrolysis of proteins generally leads to the release of peptides with low molecular weights and free amino acids[9]. According to a previous study[20], dietary proteins rich in peptides smaller than 1000 Da tend to be more reactive and contribute more effectively to flavor development, as these peptides and amino acids serve as key precursors in Maillard reactions. Such hydrolysis-derived amino acids and short peptides have been reported to yield distinct flavor profiles, with glutamic acid- and glutamate-rich oligopeptides particularly enhancing umami taste perception[9]. In our study, a substantial proportion of the hydrolysates consisted of low molecular weight peptides (<1000 Da), accounting for 62.6, 55.3, and 62.6% for mung bean, soybean, and pea, respectively. These findings are in agreement with previous results[21], who observed similar distributions in soy protein hydrolysates, with 74.83% falling in the 200–1000 Da range and 19.57% in the 1000–5000 Da range.

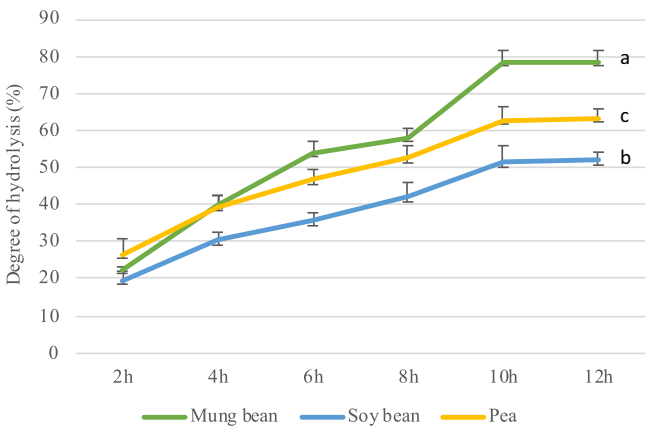


Fig. 1 | Degree of hydrolysis of the enzyme-hydrolyzed vegetable proteins: **a** Mung bean; **b** Soybean; **c** Pea.

Hydrolysates' free amino acids composition

As shown in Table 2, a total of 18 free amino acids were detected and quantified in the three hydrolysate samples. The predominant free amino acids identified in the hydrolysates included arginine, leucine, lysine, phenylalanine, and valine; this finding aligned with those of previous studies, which showed that legume proteins are rich in lysine[22] and that a relatively

Table 1 | Molecular weight distribution of enzyme-hydrolyzed vegetable proteins

MW (Da)	Mung bean	Soybean	Pea
>5000	0.54 ± 0.11	1.54 ± 0.16	ND
2000–5000	10.2 ± 0.20	20.3 ± 0.18	11.1 ± 0.25
1000–2000	26.6 ± 0.25	22.8 ± 0.25	26.3 ± 0.13
500–1000	12.0 ± 0.13	16.2 ± 0.22	16.1 ± 0.08
300–500	25.8 ± 0.28	18.9 ± 0.12	21.8 ± 0.09
100–300	24.8 ± 0.50	20.2 ± 0.34	24.7 ± 0.13

Da daltons, *MW* molecular weight, *ND* not determined.

Table 2 | Free amino acid composition of the enzyme-hydrolyzed vegetable proteins

Amino acids (μg/mL)	Mung bean	Soybean	Pea
Arg	203 ± 1.21	205 ± 0.37	269 ± 1.43
Asn/Asp	184 ± 1.10	165 ± 0.32	177 ± 0.82
Cys	2.02 ± 0.01	6.47 ± 0.01	4.52 ± 0.06
Gln/Glu	175 ± 0.50	81.6 ± 0.20	105 ± 0.57
Gly	23.2 ± 0.38	21.8 ± 0.14	25.0 ± 0.39
His	75.5 ± 0.40	65.7 ± 0.12	60.0 ± 0.30
Ile	203 ± 0.82	155 ± 0.42	191 ± 1.26
Leu	360 ± 1.62	286 ± 0.73	365 ± 2.62
Lys	313 ± 1.34	325 ± 1.21	412 ± 2.55
Met	54.6 ± 0.38	48.1 ± 0.05	42.2 ± 0.25
Phe	238 ± 1.09	156 ± 0.40	173 ± 1.03
Pro	197 ± 1.08	133 ± 0.26	217 ± 1.07
Ser	195 ± 1.41	147 ± 0.25	145 ± 1.26
Thr	110 ± 0.65	113 ± 0.17	111 ± 0.60
Tryp	36.6 ± 0.16	36.5 ± 0.08	30.5 ± 0.21
Tyr	160 ± 1.90	134 ± 0.22	143 ± 0.78
Val	294 ± 1.05	197 ± 0.48	243 ± 1.31

Arg arginine, *Asn* asparagine, *Asp* aspartic acid, *Cys* cysteine, *Gln* glutamine, *Glu* glutamic acid, *Gly* glycine, *His* histidine, *Ile* isoleucine, *Leu* leucine, *Lys* lysine, *Met* methionine, *Phe* phenylalanine, *Pro* proline, *Ser* serine, *Thr* threonine, *Tryp* Tryptophan, *Tyr* tyrosine, *Val* valine.

high content of arginine is typical for soy hydrolysates[23]. In addition, the levels of hydrophobic amino acids, such as leucine, phenylalanine, and valine, were found to be elevated in both pea and mung bean hydrolysates[24,25].

In addition, Fuentes found that Alcalase preferentially cleaves peptide bonds adjacent to hydrophobic amino acids, resulting in elevated levels of these residues in enzyme-treated protein hydrolysates[26]. Additionally, Flavourzyme was shown to increase the release of aromatic amino acids, which aligns with the present study's finding of increased phenylalanine and tyrosine concentrations. Moreover, aromatic amino acids can induce diverse flavor components through the Maillard reaction. In particular, lysine, leucine, and valine produce caramel-like odorants, whereas phenylalanine and tyrosine lead to a rose-like aroma, and arginine produces a fruity and sour aroma[27].

The contents of each free amino acids were different depending on the enzyme-hydrolyzed vegetable proteins, although most free amino acids were found in all the samples (Table 2). In particular, similar to most legume proteins, the EVPs included in this study were low in sulfur-containing amino acids; this was confirmed by the low levels of methionine and cysteine compared with other amino acids[8].

Aaslyng found that the amount of free glutamine decreased in enzyme-hydrolyzed soy protein[28]; they attributed this to the formation of a ring structure or deamidation, leading to the formation of pyroglutamic and glutamic acids. Glutamic acid, a well-known flavor-contributing amino acid, is a key factor in the distinctive taste of fermented soy sauce[29]. In our study, the contents of free glutamic acid/glutamine were the highest in mung bean, followed by pea and soybean (Table 2).

The amino acid profile is an important factor influencing the flavor characteristics of Maillard-derived compounds, with certain amino acids acting as primary precursors of meat-like flavors[5,30]. According to the previous study[30], individual amino acids exhibit different reactivities for the interactions with reducing sugars, leading to those characteristic profiles of volatile compounds. Accordingly, the specific types of amino acids present are critical to the development of volatile flavor molecules generated during Maillard reactions.

Volatile compounds formed in the hydrolysates by the Maillard reaction

The volatile compounds found in the EVPs thermally reacted with reducing sugars, along with their relative peak areas and RI values, are listed in Table 3. A total of 128 volatile compounds were identified and quantified, including 16 acids, 8 alcohols, 14 aldehydes, 1 benzene derivative, 8 esters, 44 heterocyclic compounds, 2 hydrocarbons, 17 ketones, and 14 sulfur-containing compounds. The general pathways responsible for the formation of aroma compounds during the Maillard reaction have been extensively described in previous studies, including the generation of Strecker aldehydes, nitrogen-containing heterocycles, sulfur compounds, and furan derivatives[13,31–34].

Effect of sugar types on the formation of volatile compounds

The results of the PCA plots are presented in Fig. 2. The PCA plots' total variance was 57.6%, which was 34.5% for component 1 and 23.1% for component 2. This level of explained variance, which was considered to be acceptable for exploratory analysis of complex food matrices[35], would be sufficient to clearly discriminate samples based on their compositional and volatile characteristics. Most volatile compounds were commonly detected in the glucose and fructose models; however, more ketones, aldehydes, and furans were detected in the glucose models. In contrast, pyrazines were found at significantly higher levels in the fructose models. Göğüş et al. reported that fructose exhibits greater reactivity than glucose, making it more effective in generating pyrazines during thermal reactions[36]. Therefore, in this study, the fructose models' increased formation of pyrazines may have contributed to the differences seen between them and the glucose models. In the fructose and glucose models' biplots, some pyrazines, including 2,3,5-trimethylpyrazine and 2,5-dimethylpyrazine, showed varied importance and differences between the plots. In addition, some pyrroles, including 1-(1H-pyrrol-2-yl)ethenone and 1-methylpyrrole-2-carbaldehyde, and some acids, including acetic and pentanoic acid, were the main volatile compounds that contributed to the differentiation.

Effect of the hydrolysate type on its volatile compounds

In the variable importance biplots of models evaluated, the mung bean and soy model systems could be separated by the content of sulfur-containing compounds, including 1-methylsulfanylpropane and (methyltrisulfanyl)methane. The sulfur-containing compounds, such as 1-methylsulfanylpropane and (methyltrisulfanyl)methane, were related to mung bean model systems, whereas ketones, such as 1-hydroxybutan-2-one and hexane-2,5-dione, were associated with the pea model systems. The soybean and pea model systems' PCA model biplots were distinguished by the ketones present, such as 3-hydroxybutan-2-one and 5-methylhexan-2-one.

This separation can be explained by differences in amino acid composition among the EVPs. Sulfur-containing volatiles are known to originate primarily from sulfur-containing amino acids, such as methionine, through Strecker degradation and subsequent thermal reactions during the Maillard reaction[37]. In this study, mung bean EVPs contained relatively higher levels of methionine compared with soybean and pea EVPs, which likely contributed to the stronger association of sulfur-containing compounds with the mung bean model systems.

In contrast, those ketones contributing to the separation of pea samples from others could be mainly formed through sugar degradation pathways and also interactions with amino acids and peptides during thermal processing[38]. However, the clear relationship between those ketones and the profiles of amino acids and peptides remains questionable. The complex reaction pathways between reducing sugars and amino groups from various precursors would lead to the different formations of ketones during the Maillard reaction.

Strecker aldehydes derived from amino acid degradation

Strecker degradation, a key Maillard reaction pathway, involves interactions between dicarbonyl compounds and amino acids, resulting in deamination and decarboxylation steps that significantly impact flavor formation[39]. This process generates Strecker aldehydes, which are responsible for significant odor activity, and vary depending on the amino acid profiles of the EVPs. In this study, Strecker aldehydes, such as phenylacetaldehyde and methional, both known for their roles in meat flavor formation, were found when the EVPs were reacted with reducing sugars.

Phenylacetaldehyde, which has flowery and rose-like aroma notes, is a product of phenylalanine degradation by nonenzymatic processes[40]. Phenylalanine was identified in all EVP samples, with the highest concentration observed in mung bean, followed by pea and soybean. In the glucose model systems, the Maillard reaction resulted in greater phenylacetaldehyde formation in the mung bean compared with the soybean and pea EVPs. In the fructose model systems, the mung bean had the highest phenylacetaldehyde concentration, followed by the pea and then the soybean EVPs. This finding suggests that phenylacetaldehyde formation is closely linked to the concentration of phenylalanine in EVP samples. Methional, which is associated with roasted and cooked potato aroma, and originates from methionine[33,41], was more abundant in the mung bean compared with pea and soybean EVPs. Correspondingly, methional levels were highest in the glucose model of the mung bean, followed by the pea and soybean EVPs.

Generation of nitrogen-containing heterocyclic compounds

In EVP model systems, the formation of nitrogenous heterocycles such as pyrazines, pyridines, and pyrroles through the Maillard reaction contributes significantly to the development of characteristic meaty flavors[42]. Pyrazines are produced through pathways involving α-aminoketones, which are intermediates formed from the condensation of dicarbonyl and amino compounds during the Maillard reaction[13]. In general, pyrazines produced during the Maillard reaction present roasted, burnt, and baked flavors and aromas; therefore, these compounds are crucial for evaluating the flavor and aroma quality of foods, such as coffee and meat[43].

Table 3 | Volatile compounds identified in the enzyme-hydrolyzed vegetable proteins were thermally reacted with reducing sugars

NO[a]	RI[b]	Compound	Relative peak area[c]						ID[d]
			MG	SG	PG	MF	SF	PF	
		Acids							
A1	1433	acetic acid	2.850 ± 0.641	3.300 ± 0.638	3.950 ± 0.303	0.462 ± 0.093	0.947 ± 0.551	0.015 ± 0.004	A
A2	1485	formic acid	0.107 ± 0.032	0.107 ± 0.033	0.127 ± 0.023	0.025 ± 0.012	0.083 ± 0.058	0.068 ± 0.019	A
A3	1526	propanoic acid	0.076 ± 0.013	0.114 ± 0.008	0.124 ± 0.002	0.014 ± 0.001	0.020 ± 0.005	0.059 ± 0.012	A
A4	1558	2-methylpropanoic acid	0.046 ± 0.008	ND[(5)]	0.068 ± 0.006	ND	0.014 ± 0.002	0.038 ± 0.002	A
A5	1620	prop-2-enoic acid	ND	0.051 ± 0.002	ND	ND	ND	ND	C
A6	1661	3-methylbutanoic acid	0.322 ± 0.062	0.516 ± 0.122	0.993 ± 0.072	0.104 ± 0.008	0.132 ± 0.015	1.060 ± 0.121	A
A7	1728	pentanoic acid	0.045 ± 0.010	0.044 ± 0.019	0.028 ± 0.003	ND	ND	ND	A
A8	1784	3-methylpentanoic acid	0.023 ± 0.004	0.026 ± 0.002	0.030 ± 0.003	ND	ND	0.021 ± 0.004	C
A9	1794	4-methylpentanoic acid	ND	0.189 ± 0.018	ND	ND	ND	ND	A
A10	1836	hexanoic acid	0.271 ± 0.127	0.227 ± 0.057	0.641 ± 0.055	0.030 ± 0.015	0.085 ± 0.030	0.201 ± 0.030	A
A11	1943	heptanoic acid	0.055 ± 0.010	0.035 ± 0.012	ND	0.004 ± 0.001	ND	0.016 ± 0.002	A
A12	2052	octanoic acid	0.138 ± 0.057	0.048 ± 0.030	0.018 ± 0.003	0.020 ± 0.011	0.013 ± 0.003	0.027 ± 0.006	A
A13	2128	(2E,4E)-hexa-2,4-dienoic acid	ND	ND	ND	ND	ND	0.005 ± 0.001	A
A14	2162	decanoic acid	ND	ND	ND	ND	ND	0.006 ± 0.001	A
A15	2162	nonanoic acid	0.115 ± 0.043	ND	0.011 ± 0.002	0.017 ± 0.010	ND	ND	A
A16	2405	benzoic acid	0.043 ± 0.005	0.056 ± 0.009	ND	ND	ND	ND	A
		Alcohols							
AL1	1037	2-methylbutan-1-ol	ND	ND	ND	ND	ND	0.107 ± 0.013	A
AL2	1251	pentan-1-ol	ND	0.047 ± 0.002	0.024 ± 0.009	ND	ND	0.011 ± 0.006	A
AL3	1318	(Z)-pent-2-en-1-ol	ND	0.021 ± 0.002	ND	ND	ND	ND	B
AL4	1352	1-prop-2-enoxypropan-2-ol	ND	ND	0.032 ± 0.003	ND	ND	ND	C
AL5	1356	hexan-1-ol	ND	0.198 ± 0.001	ND	ND	ND	ND	A
AL6	1594	4-methyldecan-5-ol	ND	ND	0.012 ± 0.003	ND	ND	ND	C
AL7	1866	phenylmethanol	0.010 ± 0.003	0.013 ± 0.001	0.010 ± 0.002	0.008 ± 0.001	0.005 ± 0.002	0.008 ± 0.002	A
AL8	1903	2-phenylethanol	ND	ND	ND	0.012 ± 0.001	ND	ND	A
		Aldehydes							
AD1	1084	(E)-2-methylbut-2-enal	ND	ND	ND	0.318 ± 0.070	0.096 ± 0.011	0.121 ± 0.005	A
AD2	1085	(E)-4-phenylbut-2-enal	ND	ND	ND	ND	0.009 ± 0.007	ND	B
AD3	1105	hexanal	0.018 ± 0.006	ND	ND	ND	0.123 ± 0.078	0.111 ± 0.039	A
AD4	1138	(2E,4E)-octa-2,4-dienal	0.009 ± 0.001	ND	ND	ND	ND	ND	C
AD5	1192	3-methylbut-2-enal	0.149 ± 0.032	0.094 ± 0.053	0.106 ± 0.009	0.230 ± 0.053	0.096 ± 0.028	0.092 ± 0.004	A
AD6	1299	octanal	0.100 ± 0.019	ND	ND	0.048 ± 0.030	ND	ND	A
AD7	1375	(E)-5--2-propan-2-ylhex-2-enal	ND	ND	ND	ND	ND	0.077 ± 0.046	A
AD8	1516	benzaldehyde	1.190 ± 0.494	2.190 ± 0.267	1.150 ± 0.143	1.840 ± 0.224	1.010 ± 0.241	1.070 ± 0.303	A
AD9	1633	2-phenylacetaldehyde	0.946 ± 0.142	0.870 ± 0.127	0.542 ± 0.085	0.633 ± 0.110	0.249 ± 0.107	0.482 ± 0.155	A
AD10	1669	3-methylbutanal	ND	ND	0.008 ± 0.001	ND	ND	ND	A
AD11	1670	4-hydroxybenzaldehyde	0.017 ± 0.006	ND	ND	ND	ND	ND	A
AD12	1773	3-phenylpropanal	ND	ND	ND	0.007 ± 0.001	ND	ND	B
AD13	1855	5-methyl-2-furaldehyde	ND	ND	ND	ND	ND	0.010 ± 0.003	A
AD14	1923	(E)-2-phenylbut-2-enal	0.051 ± 0.007	0.059 ± 0.015	0.051 ± 0.010	0.021 ± 0.002	ND	0.026 ± 0.013	A
		Benzene derivatives							
B1	1037	toluene	ND	0.195 ± 0.016	ND	ND	ND	ND	A
		Esters							
E1	1037	butyl 2-methylpropanoate	ND	ND	ND	ND	0.091 ± 0.024	ND	A
E2	1228	methyl 2-oxopropanoate	ND	ND	ND	ND	0.022 ± 0.004	ND	C
E3	1357	3-methylbutyl formate	0.027 ± 0.012	ND	ND	ND	ND	ND	A
E4	1369	methyl acetate	0.043 ± 0.002	ND	ND	ND	ND	ND	A
E5	1477	methyl 3,3-dimethyl-6-oxohept-4-enoate	ND	ND	ND	ND	ND	0.022 ± 0.008	C
E6	1785	2-methylpropyl formate	ND	ND	ND	ND	0.013 ± 0.000	ND	C

Table 3 (continued) | Volatile compounds identified in the enzyme-hydrolyzed vegetable proteins were thermally reacted with reducing sugars

NO[a]	RI[b]	Compound	Relative peak area[c]						ID[d]
			MG	SG	PG	MF	SF	PF	
E7	1841	2,3-dihydropyran-6-one	0.022 ± 0.007	0.024 ± 0.007	0.022 ± 0.009	ND	ND	0.011 ± 0.001	A
E8	1906	[2,2,4-trimethyl-3-(2-methylpropanoyloxy) pentyl] 2-methylpropanoate	ND	ND	0.012 ± 0.001	ND	0.007 ± 0.001	ND	A
		Furans & Furanones							
F1	1242	2-pentylfuran	0.123 ± 0.056	ND	0.032 ± 0.008	0.239 ± 0.017	0.060 ± 0.051	0.052 ± 0.024	A
F2	1423	5-methyl-3H-furan-2-one	0.010 ± 0.002	0.014 ± 0.003	0.018 ± 0.004	ND	0.010 ± 0.002	0.018 ± 0.005	A
F3	1454	furan-2-carbaldehyde	4.930 ± 2.950	8.080 ± 0.684	4.000 ± 0.952	1.360 ± 0.258	0.812 ± 0.519	0.433 ± 0.046	A
F4	1478	2,3,5-trimethylfuran	ND	ND	0.023 ± 0.007	ND	ND	ND	C
F5	1496	1-(furan-2-yl)ethenone	0.565 ± 0.085	1.070 ± 0.036	0.776 ± 0.146	0.188 ± 0.031	0.131 ± 0.073	0.376 ± 0.139	A
F6	1531	furan-2-ylmethyl acetate	ND	0.010 ± 0.002	0.009 ± 0.001	ND	ND	0.010 ± 0.002	A
F7	1555	5-methylfuran-2-carbaldehyde	0.686 ± 0.102	2.020 ± 0.155	1.180 ± 0.224	0.045 ± 0.012	0.047 ± 0.026	0.185 ± 0.054	A
F8	1650	furan-2-ylmethanol	1.080 ± 0.179	1.920 ± 0.229	2.320 ± 0.406	0.946 ± 0.118	1.100 ± 0.481	2.580 ± 0.363	A
F9	1656	3,4-dimethyl-2H-furan-5-one	0.006 ± 0.001	ND	ND	ND	ND	ND	C
F10	1713	(5-methylfuran-2-yl)methanol	0.082 ± 0.006	0.076 ± 0.028	0.051 ± 0.011	0.077 ± 0.008	ND	ND	A
F11	1736	2H-furan-5-one	0.019 ± 0.008	0.021 ± 0.003	0.023 ± 0.003	ND	0.073 ± 0.012	0.051 ± 0.011	A
F12	1765	methylfuran-2-carboxylate	ND	ND	ND	ND	ND	0.015 ± 0.002	C
F13	2019	4-hydroxy-2,5-dimethylfuran-3-one	0.012 ± 0.003	ND	0.009 ± 0.001	0.005 ± 0.002	0.005 ± 0.002	0.015 ± 0.009	A
F14	2477	5-(hydroxymethyl)furan-2-carbaldehyde	0.061 ± 0.001	0.053 ± 0.018	0.033 ± 0.014	ND	ND	0.021 ± 0.013	A
		Hydrocarbons							
H1	1149	7,9-dimethylhexadecane	0.012 ± 0.002	ND	ND	ND	ND	ND	C
H2	1349	(2E,4E)-hexa-2,4-diene	0.083 ± 0.016	0.054 ± 0.003	ND	ND	ND	ND	C
		Ketones							
K1	1052	pentane-2,3-dione	ND	0.467 ± 0.129	0.239 ± 0.073	0.238 ± 0.063	0.121 ± 0.069	0.143 ± 0.055	A
K2	1052	2-methylpentan-3-one	0.223 ± 0.074	ND	ND	ND	ND	ND	A
K3	1143	5-methylhexan-2-one	ND	ND	0.057 ± 0.017	ND	ND	0.009 ± 0.004	A
K4	1148	heptane-2,3-dione	ND	0.045 ± 0.022	ND	0.051 ± 0.044	ND	0.013 ± 0.005	C
K5	1153	2-methylheptan-3-one	0.011 ± 0.002	ND	ND	ND	ND	ND	C
K6	1185	heptan-2-one	0.025 ± 0.006	ND	ND	ND	0.016 ± 0.006	ND	A
K7	1277	3-hydroxybutan-2-one	0.077 ± 0.018	0.091 ± 0.005	0.136 ± 0.047	0.036 ± 0.002	0.077 ± 0.030	0.133 ± 0.035	A
K8	1283	octane-2,3-dione	0.136 ± 0.016	0.300 ± 0.078	0.227 ± 0.068	0.418 ± 0.033	0.159 ± 0.110	0.299 ± 0.191	C
K9	1289	1-hydroxypropan-2-one	1.590 ± 0.329	1.670 ± 0.174	1.910 ± 0.232	0.778 ± 0.053	1.210 ± 0.267	1.650 ± 0.273	A
K10	1364	1-hydroxybutan-2-one	0.027 ± 0.004	0.044 ± 0.002	0.045 ± 0.007	0.023 ± 0.001	0.020 ± 0.008	0.043 ± 0.006	A
K11	1572	cyclopent-4-ene-1,3-dione	0.169 ± 0.044	0.288 ± 0.018	0.161 ± 0.029	0.035 ± 0.010	0.023 ± 0.010	0.034 ± 0.020	A
K12	1610	1-(2-ethyloxiran-2-yl)ethenone	0.031 ± 0.006	ND	ND	ND	ND	ND	C
K13	1610	hexane-2,5-dione	ND	0.058 ± 0.015	0.042 ± 0.002	ND	0.013 ± 0.007	0.031 ± 0.007	C
K14	1722	1-phenylpropan-2-one	0.018 ± 0.004	0.028 ± 0.004	0.021 ± 0.003	ND	ND	0.029 ± 0.007	A
K15	1766	(3E,5E)-hepta-3,5-dien-2-one	ND	ND	0.017 ± 0.001	ND	ND	ND	C
K16	1817	3-methylcyclopentane-1,2-dione	ND	ND	ND	0.009 ± 0.001	ND	ND	C
K17	1817	2-hydroxy-3-methylcyclopent-2-en-1-one	0.066 ± 0.018	0.091 ± 0.005	0.114 ± 0.017	ND	0.016 ± 0.003	0.040 ± 0.012	A
		Lactones							
L1	1258	2-methyloxolan-3-one	0.269 ± 0.047	0.484 ± 0.040	0.361 ± 0.067	0.024 ± 0.005	0.053 ± 0.022	0.092 ± 0.009	A
L2	1613	oxolan-2-one	0.019 ± 0.005	0.011 ± 0.003	0.030 ± 0.004	0.022 ± 0.005	0.017 ± 0.010	0.054 ± 0.017	A
L3	2250	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	0.114 ± 0.009	0.037 ± 0.020	0.027 ± 0.010	0.046 ± 0.019	0.028 ± 0.011	0.075 ± 0.064	B
		Phenols							
P1	1990	phenol	0.024 ± 0.007	0.022 ± 0.001	0.025 ± 0.003	0.018 ± 0.003	0.011 ± 0.004	0.020 ± 0.004	A
		Pyrazines							
PZ1	1263	2-methylpyrazine	1.700 ± 0.311	0.956 ± 0.106	0.600 ± 0.265	4.820 ± 0.943	2.460 ± 1.230	3.710 ± 1.380	A
PZ2	1323	2,5-dimethylpyrazine	0.316 ± 0.054	0.514 ± 0.065	0.419 ± 0.068	11.200 ± 1.370	7.200 ± 1.640	9.840 ± 5.030	A
PZ3	1330	2,6-dimethylpyrazine	0.050 ± 0.011	0.083 ± 0.017	0.069 ± 0.013	7.760 ± 1.730	4.090 ± 0.886	5.250 ± 1.600	A
PZ4	1335	2-ethylpyrazine	ND	ND	0.125 ± 0.037	ND	ND	ND	A
PZ5	1347	2,3-dimethylpyrazine	ND	ND	ND	0.223 ± 0.055	0.087 ± 0.034	0.152 ± 0.065	A

Table 3 (continued) | Volatile compounds identified in the enzyme-hydrolyzed vegetable proteins were thermally reacted with reducing sugars

NO[a]	RI[b]	Compound	Relative peak area[c]						ID[d]
			MG	SG	PG	MF	SF	PF	
PZ6	1396	2-ethyl-5-methylpyrazine	0.012 ± 0.001	0.027 ± 0.002	0.019 ± 0.004	0.173 ± 0.019	0.138 ± 0.076	0.197 ± 0.106	B
PZ7	1397	2-ethyl-6-methylpyrazine	ND	0.056 ± 0.001	0.019 ± 0.001	0.089 ± 0.005	0.049 ± 0.039	0.074 ± 0.049	B
PZ8	1408	2,3,5-trimethylpyrazine	0.078 ± 0.014	0.081 ± 0.005	0.053 ± 0.011	0.616 ± 0.061	0.485 ± 0.251	0.586 ± 0.300	A
PZ9	1419	2-methyl-5-propan-2-ylpyrazine	ND	ND	ND	0.034 ± 0.007	ND	ND	B
PZ10	1433	2-ethenylpyrazine	ND	ND	ND	0.028 ± 0.002	ND	ND	B
PZ11	1470	3-ethyl-2,5-dimethylpyrazine	ND	ND	ND	ND	0.015 ± 0.003	0.021 ± 0.009	B
PZ12	1489	2-ethenyl-6-methylpyrazine	0.036 ± 0.010	0.040 ± 0.003	0.033 ± 0.005	0.137 ± 0.012	0.082 ± 0.035	0.105 ± 0.038	B
PZ13	1543	2,3-diethyl-5-methylpyrazine	ND	ND	ND	0.038 ± 0.001	ND	0.021 ± 0.012	C
PZ14	1543	2-ethenyl-6-ethylpyrazine	ND	ND	ND	ND	0.021 ± 0.011	ND	C
PZ15	1620	1-pyrazin-2-ylethanone	ND	ND	0.077 ± 0.004	0.031 ± 0.004	ND	0.053 ± 0.007	B
PZ16	1678	2,5-dimethyl-3-(3-methylbutyl)pyrazine	ND	ND	ND	0.155 ± 0.010	ND	ND	C
PZ17	1679	2-butyl-3,5-dimethylpyrazine	ND	ND	ND	ND	0.114 ± 0.046	ND	C
PZ18	1689	1-(3-methylpyrazin-2-yl)ethenone	ND	0.017 ± 0.002	0.016 ± 0.002	0.026 ± 0.004	0.027 ± 0.008	0.039 ± 0.012	C
		Pyridines							
PD1	1179	pyridine	0.091 ± 0.029	ND	ND	0.103 ± 0.039	0.036 ± 0.037	0.041 ± 0.017	A
PD2	1554	1H-pyridin-2-one	0.025 ± 0.013	ND	ND	ND	ND	ND	C
PD3	1597	1-pyridin-2-ylethanone	ND	0.018 ± 0.001	0.018 ± 0.002	ND	ND	ND	A
PD4	1597	1-pyridin-3-ylethanone	0.012 ± 0.002	ND	ND	ND	ND	ND	C
PD5	1669	(NE)-*N*-(pyridin-2-ylmethylidene)hydroxylamine	ND	ND	ND	0.020 ± 0.007	ND	ND	C
PD6	1766	(3E,5E)-hepta-3,5-dien-2-one	ND	ND	0.017 ± 0.001	ND	ND	ND	C
		Pyrroles							
PR1	1499	1-(furan-2-ylmethyl)pyrrole	ND	ND	ND	ND	0.039 ± 0.014	ND	A
PR2	1888	1-methylpyrrole	0.021 ± 0.011	ND	ND	ND	ND	ND	A
PR3	1957	1-(1H-pyrrol-2-yl)ethenone	0.475 ± 0.084	0.486 ± 0.109	0.568 ± 0.089	0.040 ± 0.009	0.030 ± 0.009	0.077 ± 0.012	A
PR4	2008	1H-pyrrole-2-carbaldehyde	0.017 ± 0.005	0.021 ± 0.003	0.020 ± 0.003	0.010 ± 0.003	0.008 ± 0.004	0.017 ± 0.002	A
PR5	2085	1-methylpyrrole-2-carbaldehyde	0.026 ± 0.006	0.043 ± 0.005	0.045 ± 0.008	ND	0.004 ± 0.000	0.008 ± 0.002	A
PR6	2086	5-methyl-1H-pyrrole-2-carbaldehyde	ND	ND	ND	ND	ND	0.010 ± 0.002	A
		Sulfur-containing compounds							
S1	1062	(methyldisulfanyl)methane	1.980 ± 0.319	1.960 ± 0.331	3.210 ± 0.660	2.030 ± 0.499	0.723 ± 0.094	0.469 ± 0.147	A
S2	1158	3-methylthiophene-2-carboxylic acid	0.014 ± 0.003	ND	ND	ND	ND	ND	C
S3	1236	1-methylsulfanylpropane	0.068 ± 0.012	ND	ND	0.066 ± 0.015	ND	ND	B
S4	1242	1,3-thiazole	ND	0.062 ± 0.013	ND	ND	ND	ND	A
S5	1251	1-(methyldisulfanyl)propane	0.021 ± 0.008	ND	ND	ND	ND	ND	A
S6	1379	(methyltrisulfanyl)methane	0.985 ± 0.658	0.991 ± 0.124	0.674 ± 0.127	1.220 ± 0.165	0.327 ± 0.047	0.263 ± 0.126	A
S7	1448	3-methylsulfanylpropanal	0.955 ± 0.245	0.836 ± 0.513	0.431 ± 0.083	0.754 ± 0.123	0.358 ± 0.120	1.840 ± 0.339	A
S8	1500	2-(methylsulfanylmethyl)furan	ND	ND	ND	0.078 ± 0.014	ND	0.043 ± 0.025	B
S9	1554	thiolan-3-one	ND	ND	ND	ND	0.011 ± 0.009	ND	A
S10	1656	4-methyl-1,2-thiazole	0.004 ± 0.000	ND	ND	ND	ND	ND	A
S11	1720	3-methylthiophene-2-carbaldehyde	ND	ND	ND	0.028 ± 0.002	ND	ND	A
S12	1721	5-methylthiophene-2-carbaldehyde	ND	ND	ND	ND	0.019 ± 0.009	ND	A
S13	1766	2-ethyl-5-methylthiophene	ND	ND	ND	ND	0.012 ± 0.003	ND	A
S14	2304	imidazolidine-2-thione	0.005 ± 0.000	ND	ND	ND	ND	ND	A

ND not detected, *MG* mung bean with glucose, *MF* mung bean with fructose, *SG* soybean with glucose, *SF* soybean with fructose, *PG* pea with glucose, *PF* pea with fructose.

[a]All volatile compounds are listed according to the order of their RI values within the chemical class.

[b]Using the n-alkanes C_7–C_{30} as external standards, retention indices were calculated.

[c]Average values of the relative peak area to that of the internal standard ± standard deviation ($n = 3$).

[d]The following standards were used to identify compounds: A, the relative retention index and mass spectrum matched with those of the authentic standard compounds under identical conditions (positive identification); B, the relative retention index (RI) and mass spectrum matched with those found in the NIST database (tentative identification); and C, the mass spectrum that agreed with that of the W9N08 (Wiley and NIST) and manual interpretation (tentative identification).

Wang et al[44] found that arginine promotes the formation of 2-ethenyl-6-methylpyrazine. The highest level of arginine was found in the pea hydrolysate. In addition, the concentration of 2-ethenyl-6-methylpyrazine was highest in the pea-fructose model. Additionally, 2-methylpyrazine, which has popcorn-like aromas, is formed in the presence of arginine, histidine, and lysine[45], and was detected in all the model systems, with the pea-fructose model yielding higher levels than the soybean model.

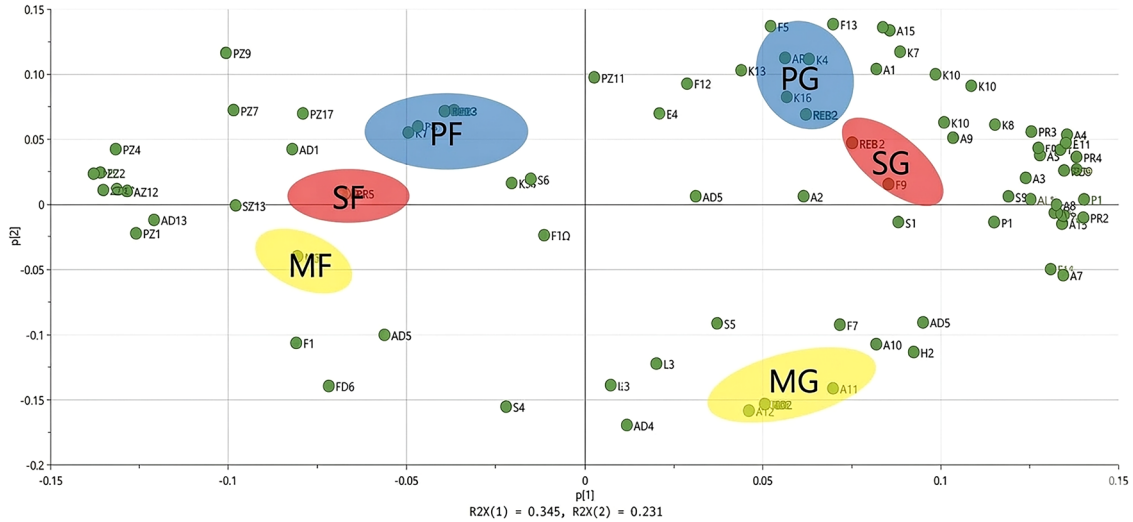


Fig. 2 | Principal component analysis plots of the hydrolysates' volatile compounds from the Maillard reaction. MG mung bean with glucose, MF mung bean with fructose, SG soybean with glucose, SF soybean with fructose, PG pea with glucose, PF pea with fructose.

Pyridine compounds can be formed via condensation of ketones, α, β-unsaturated carbonyls, or aldehydes, with ammonia, the latter being a byproduct of amino acid breakdown[46]. According to ref. 47, 2-acetylpyridine is associated with proline and glycine. The pea EVP's proline and glycine concentrations were higher than those of the soybean EVP, leading to a greater amount of 2-acetylpyridines detected in the pea-glucose model compared to the soybean-glucose model. In contrast, ref. 48 demonstrated that 2-hydroxypyridine was only identified after the reaction of asparagine with glucose. This finding is in agreement with this study's findings, as 2-hydroxypyridine was only detected in the mung bean-glucose model; the mung bean EVP's asparagine level was highest between the hydrolysates.

Formation of sulfur-containing aroma

Sulfur-containing flavor compounds typically possess low detection thresholds and unique olfactory properties, playing an important role in the perception of cooked-meat flavor in numerous food matrices[45]. Accordingly, cysteine and methionine serve as key precursors in generating meat-like aroma compounds during thermal reactions, including the Maillard reaction and Strecker degradation[49,50]. In general, despite the relatively low abundance of sulfur-containing amino acids, those related sulfur volatiles were quantified at comparatively high levels in our study. This may be attributed to the high reactivity of sulfur-containing amino acids, degrading into diverse sulfur volatiles readily.

Some volatile sulfur compounds with low molecular weights, including dimethyl disulfide and dimethyl trisulfide, are known to impart onion- and garlic-like odor notes[51]. According to ref. 37, when methionine reacts with reducing sugars like glucose, it leads to the production of diverse sulfur volatiles such as dimethyl sulfide and dimethyl disulfide. This study found that the content of methionine was highest in the mung bean, followed by the soybean and pea EVPs; moreover, the levels of dimethyl disulfide were highest in the mung bean-fructose, followed by the soybean-fructose and pea-fructose models. Dimethyl trisulfide, a sulfur-containing compound derived from methionine, had the highest concentration in the mung bean, followed by soybean and pea EVPs in the fructose models. A similar tendency was found for dimethyl disulfide in the glucose models, with the mung bean showing higher levels than the pea model[49].

Furan derivatives generated from sugar degradation

Thermal decomposition of sugars is known to generate heterocyclic furan derivatives, which have been widely detected in numerous food matrices[52]. In particular, 5-hydroxymethylfurfural and furfural, which exhibit characteristic caramel and fruity aroma notes, are primary degradation products of sugars[38,53]. Overall, these two compounds were found at higher levels in the mung bean than in the pea models, with furfural showing particularly high concentrations in the glucose models. While these compounds can positively contribute to sweetness-associated aroma characteristics at moderate levels, excessive accumulation has been associated with undesirable sensory attributes such as burnt or bitter notes[38]. Therefore, their formation should be carefully controlled to achieve a balanced flavor profile during thermal processing.

Furaneol, a volatile compound formed through the thermal degradation of fructose, has been identified in diverse e model systems involving the breakdown of both fructose and glucose[38]. Due to its thermal lability, furaneol readily decomposes into various carbonyl and hydroxy-carbonyl compounds, including 2-hydroxy-3-butanone and 2-hydroxy-3-pentanone under high temperatures[38]. In this study, furaneol and its degradation product, 2-hydroxy-3-pentanone, demonstrated a consistent trend in the EVP samples. Specifically, the furaneol content was higher in the pea models compared with the soybean and mung bean models; 2-hydroxy-3-pentanone showed a similar trend, being detected at its highest levels in the pea models. This correlation between furaneol and its degradation product supports the hypothesis of its progressive breakdown during the Maillard reaction, reinforcing a finding from a previous study[38].

Discussion

In this study, enzyme-hydrolyzed vegetable proteins (EVPs) derived from mung bean, soybean, and pea were systematically compared under identical hydrolyzing and reaction conditions to elucidate their physicochemical characteristics and their influence on Maillard reaction-derived volatile compounds. Following enzymatic hydrolysis, all EVPs were predominantly composed of low molecular weight fractions below 1000 Da; however, clear differences were observed among the protein sources in terms of degree of hydrolysis, free amino acid composition, and subsequent volatile formation.

Compared with soybean EVP, mung bean and pea EVPs exhibited higher levels of total free amino acids, particularly, key Maillard reaction precursors such as leucine, lysine, and arginine. These compositional differences could lead to the variations in volatile profiles generated during thermal reactions with reducing sugars. Specifically, mung bean and pea EVPs produced significantly higher amounts of characteristic Maillard reaction-derived aroma compounds, including 2-methylpyrazine, 2-acetylpyridine, and 4-methyl-1,2-thiazole, which are important contributors to roasted and meat-like flavor notes. These results indicate that the intrinsic properties of EVPs play a critical role in determining the type and abundance of volatile compounds formed during the Maillard reaction.

Overall, the results demonstrate that mung bean and pea proteins, when enzymatically hydrolyzed, provide more favorable precursor profiles for Maillard reaction-driven flavor generation than soybean protein. These findings offer valuable insights that support the potential application of mung bean- and pea-derived EVPs as alternative protein ingredients in plant-based flavoring systems and processed food formulations. Further studies incorporating sensory evaluation and pilot-scale processing are warranted to confirm their practical applicability and enhance their use in industrial plant-based food products.

Methods

Materials and chemicals

Mung beans, harvested in Peru in 2023, were sourced from Adesfood (Dong-gu, Busan, Republic of Korea). Soybeans, cultivated in Korea in 2023, were purchased from Babhankki (Namyangju-si, Gyeonggi-do, Republic of Korea), while peas, cultivated in Korea in 2024, were obtained from Nongrangburang (Ansan-si, Gyeonggi-do, Republic of Korea). All raw materials were preserved at −70 °C in a deep freezer until further analysis.

Alcalase® (2.4 AU/g) and Flavourzyme® (1100 LAPU/g) were obtained from Novozymes (Bagsværd, Denmark). These enzymes were selected due to their complementary proteolytic activities. Alcalase acts primarily as an endopeptidase that promotes extensive internal peptide bond cleavage, while Flavourzyme exhibits both endo- and exopeptidase activities that further hydrolyze peptides into free amino acids and short peptides[54]. Analytical-grade reagents and chemicals used in this study were purchased from Sigma-Aldrich® (Merck KGaA, Darmstadt, Germany). The internal standards used for volatile compounds and free amino acids analysis were (-)-borneol and L-norleucine (Sigma-Aldrich®); the standard solutions were prepared in methanol. The *n*-alkane standards (C_7–C_{30}) and authentic standards were also obtained from Sigma-Aldrich®. Solvents, including methanol, acetonitrile, and HPLC-grade water, were supplied by J.T. Baker® (Avantor, Inc., Radnor, PA, USA), while hexane was purchased from Samchun Chemical Co., Ltd. (Gangnam-gu, Seoul, Republic of Korea).

Preparation of the samples

The whole mung beans, soybeans, and peas were dehulled and cleaned of the loose hulls. The dehulled beans were dried, milled using a blender (Shinil Industrial Co, Seoul, Republic of Korea), and then passed through a 100 mesh sieve. The flours were defatted using hexane in accordance with a previously reported method[55] before protein extraction. Protein extraction was conducted according to a reported procedure[56], after which the dispersion was freeze-dried and stored at −70 °C for later use.

Preparation of hydrolysates

A two-step enzymatic hydrolysis was performed on the defatted protein isolates, following a modified protocol based on a reported method[54]. Briefly, a protein solution (5% w/v) was obtained by dissolving extracted proteins in deionized water, stirred for 10 min, and then adjusted to pH 8.0 using 4 M NaOH. Alcalase was added at 1% enzyme-to-substrate ratio, and hydrolysis was carried out at 50 ± 2 °C for 1 h. The reaction was stopped by lowering the pH to 5.0 with 4 M HCl. The pH was subsequently readjusted to 8.0 using 4 M NaOH, before Flavourzyme was added under the same conditions (1% enzyme-to-substrate ratio, 50 ± 2 °C). Hydrolysis then continued for an additional 9 h. Then, enzymatic activity was terminated by adjusting the pH to 5.0. After centrifugation at 26,664×*g* for 20 min at 4 °C, the supernatants were freeze-dried and preserved at −70 °C for subsequent analyses.

Measurement of the degree of hydrolysis

The DH was determined using a modified formol titration method[57]. Specifically, 1.0 M NaOH was used as the titrant. The Kjeldahl method was used to determine the hydrolysates' crude protein content; this was performed by the Korea Advanced Food Research Institute of Korea Food Industry Association (Uiwang-si, Gyeonggi-do, Republic of Korea).

Analysis of the hydrolysates' molecular weight distribution

The molecular weight (MW) distribution analysis was conducted by Korea Polymer Testing & Research Institute, Ltd. (Seoul, Republic of Korea). The MW distribution profiles of samples were determined by gel permeation chromatography using a Tosoh HLC-8420 GPC Elite (Tosoh Co., Tokyo, Japan) with a differential refractive index detector, and TSKgel guard PWXL, TSKgel GMPWXL, and TSKgel G2500PWXL columns (Tosoh Co.). The samples (3 mg/mL) were filtered through a 0.45 μm nylon filter. The mobile phase was 0.1 M $NaNO_3$ at a temperature of 40 °C, a flow rate of 1.0 mL/min, and an injection volume of 100 μL. A MW calibration curve was obtained from the Poly(ethylene glycol)/Poly(ethylene oxide) standards.

Analysis of free amino acids

The profile of free amino acids in the hydrolysates was determined using an Agilent 1260 HPLC system equipped with a UV-visible diode array detector (Agilent Technologies Inc., Santa Clara, CA, USA) and a Gemini C_{18} column (5 μm, 250 × 4.6 mm). Sample preparation and derivatization followed the previous studies[31,58], with minor modifications. The gradient elution was carried out at 1.0 mL/min with the column temperature held at 30 °C and UV detection set to 254 nm. The gradient elution was programmed as follows: 90% solvent A (10% B) to 8 min, 75% A (25% B) to 10 min, 75% A to 12 min, 52% A (48% B) to 18 min, 52% A to 20 min, 0% A (100% B) to 25 min, and 90% A to 35 min.

Preparation of the Maillard reaction samples

Before the Maillard reaction, 1 g of each EVP hydrolysate was mixed with 0.5 g of either fructose or glucose and diluted to 10 mL using HPLC-grade water. The pH of the solution was adjusted to 7.5 with 1 M NaOH before being transferred into a 250 mL stainless steel reactor, based on a previous study indicating that Maillard reactions are normally conducted under alkaline conditions to facilitate reaction efficiency and flavor development[13] (Ilshin Autoclave Co., Ltd., Daejeon-si, Republic of Korea). Samples were subjected to thermal treatment at 160 °C for 90 min in a drying oven (Eyela NDO-600SD, Rikakikai Co. Ltd., Tokyo, Japan). Based on the combination of protein and sugar added, samples were labeled as MF, MG, SF, SG, PF, and PG (Table 4).

Analysis of the samples' volatile compounds

Volatile compounds were extracted using solid-phase microextraction (SPME) with a GERSTEL MPS Robotic autosampler (GERSTEL GmbH & Co. KG, Mülheiman der Ruhr, Germany) and a Supelco® Carboxen®/polydimethylsiloxane fiber (75 μm, Merck KGaA, Darmstadt, Germany). Each sample (5 g) was accurately weighed and sealed in a 20 mL headspace vial, followed by the addition of 10 μL of internal standard solution [(-)-borneol in methanol, 500 μg/mL], which was selected due to its absence in the samples and its stable, reproducible extraction and GC–MS response. After 30 min of equilibration at 50 °C, volatiles were adsorbed for 30 min.

Analysis was performed using an Agilent 7890A gas chromatograph coupled to a 5977B mass selective detector (Agilent Technologies Inc.) and fitted with a DB-WAX column (30 m × 0.25 mm i.d., 0.25 μm). Helium was employed as the carrier gas at a constant flow rate of 0.8 mL/min. The injector and transfer line temperatures were set to 230 and 280 °C, respectively. The oven temperature program began at 40 °C (held for 3 min), followed by increases to 80 °C at 5 °C/min, 160 °C at 10 °C/min (held for

Table 4 | Model systems comprising enzyme-hydrolyzed vegetable proteins and reducing sugars

		EVPs		
		Mung bean (M)	Soybean (S)	Pea (P)
Reducing sugars	Glucose (G)	MG	SG	PG
	Fructose (F)	MF	SF	PF

0.5 min), 175 °C at 2 °C/min, and finally 230 °C at 10 °C/min (held for 7 min). Electron impact ionization at 70 eV was applied, with mass spectra collected over 35–450 m/z.

Volatile compounds were identified by comparing their relative retention times and mass spectra with those of authentic standards. When standards were unavailable, tentative identification was carried out based on mass spectral data from the NIST/EPA/NIH Mass Spectral Library (NIST.08) (NIST Mass Spectrometry Data Center, US Department of Commerce, Gaithersburg, MD, USA) and Wiley.9 (Wiley Science Solutions, John Wiley & Sons, Inc., Hoboken, NJ, USA), along with relative retention index values. The relative retention index value was determined using a series of n-alkanes (C_7– C_{30}). The relative concentrations of the volatiles were determined by comparing their peak areas to that of the internal standard.

Data analysis

Principal component analysis (PCA) and partial least square-discrimination analysis were performed to compare the characteristics of volatile profiles between the different types of EVPs by using SIMCA® 16 software (Sartorius Stedim Biotech SA, Aubagne, France).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Y.L. conducted the investigation, curated the data, developed the methodology, and wrote the original draft of the manuscript. Y.-S.K. conceptualized the study, curated the data, supervised the research, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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