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Profiles of aroma volatile components in textured vegetable proteins using headspace solid phase microextraction-gas chromatography-mass spectrometry

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ABSTRACT

Textured vegetable protein (TVP) is a significant alternative to meat, with its primary raw materials being soybeans, peas, rice, and wheat proteins. While advancements in technology have successfully replicated the unique texture of meat in plant-based proteins, research on the aroma profiles of these key raw materials remains limited. The subtle differences in aroma between meat and meat substitutes are yet to be fully addressed. In this study, we employed headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS), a specialized technique for the analysis of volatile aromatic compounds, to examine the volatile profiles of soybean, pea, rice, and wheat proteins. The identified volatile compounds included alcohols, aldehydes, carboxylic acids, ethers, furans, indoles, ketones, phenols, pyrans, and sulfur compounds. Based on prior research, eight compounds (hexanal, nonanal, 2-nonenal, 3-methylbutanal, benzaldehyde, 1-octen-3-ol, 3-octen-2-one, and 2-pentylfuran) were classified as off-flavors. Hexanal, a key marker, was found in the following order: rice showed the highest levels, followed by soybeans, peas, and wheat. Other major volatile components exhibited distinct ratios across the samples. These findings could assist in refining the next generation of TVPs and minimizing aroma heterogeneity.

1. Introduction

Meat is a valuable source of high-quality protein, but increasing environmental concerns have driven active research into the development of alternative food sources. The plant-based alternative protein market is expanding at 7.2% annually, projected to reach $15.6 billion by 2026 (Markets and Markets). While plant-based protein products represent 2% of the global food market, it is growing rapidly, with an average annual growth of approximately 6% (Joseph et al., 2020).

In this context, plant-based meat substitutes, seaweed, edible insects, and microbially cultured meat are gaining attention as potential alternatives to conventional meat (Lee et al., 2021; Kim, 2018; You et al., 2020; Kyriakopoulou et al., 2019). However, unlike animal-based ingredients, plant proteins exhibit a wide range of compositions, physicochemical characteristics, and structural properties, which complicates efforts to replicate the texture, properties, and flavor of meat or meat products. Furthermore, plant proteins must undergo processing to transform their natural spherical structures into linear, textured forms, which is achieved through techniques such as extrusion, electrospinning, and wet spinning (Kim et al., 2017). Plant-based meat substitutes are primarily formulated using vegetable proteins from grains, legumes, seeds, nuts, and stems. Key raw materials include soy, rice, wheat gluten, pea, and mung bean proteins, with peanut and potato proteins also used (Yong et al., 2021). soybean protein, composed mainly of globulins, is valued for its high protein content and superior fat and water-binding properties, enhancing the water retention, emulsification, and texture of products like sausages and patties (Yong et al., 2021). Its cost-effectiveness further supports its popularity as a meat substitute (Kim et al., 2009a). However, soybean protein may be unsuitable for some consumers due to allergies or concerns about genetically modified organisms (GMOs).

The production of TVP involves blending proteins from rice, wheat, peas, and soybeans to leverage their individual strengths and create a high-quality product. This combination enhances texture, enabling TVP to mimic the chewiness and structure of meat (Saunders, 1990; Chanput et al., 2009). Rice protein adds a soft, light texture, wheat protein contributes elasticity and structure, and pea and soybean proteins balance the texture and enrich the overall product (Mun, 2020; Wieser, 2007).

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Table 1
Various studies on monitoring volatile flavor components in foods using solid-phase microextraction (SPME).

Samples	Title	References
Alcohols	Monitoring of the changes in volatile flavor components in oriental melon wine using SPME	Jo et al. (2013)
	Volatile analysis of commercial Korean black raspberry wines (Bokbunjaju) using headspace solid-phase microextraction	Lee (2014)
	Volatile component analysis of commercial Japanese distilled liquors (shochu) by headspace solid-phase microextraction	Shin and Lee (2015)
	Changes in volatile compounds in rice-based distilled soju aged in different types of containers	Kim and Lee (2019)
	Characterization and volatile flavor components in glutinous rice wines prepared with different yeasts of Nuruks	Kim et al. (2009b)
Coffee	Changes in aroma compounds of decaffeinated coffee beans	Lee and Kim (2023)
	Impacts of coffee creamer, dried skim milk and sugar on the volatile aroma compounds and sensory characteristics in instant coffee	Min et al. (2015)
	Development of novel spray freeze-drying method for value-added coffee powder preparation	Her (2021)
	Analysis of off-flavor compounds from over-extracted coffee	Lee et al. (2011)
Tea and flower	Comparison of flavor compounds in steamed- and nonsteamed-roasted Polygonatum odoratum roots by solid-phase microextraction	Park et al. (2000)
	Volatile flavor composition of white-flowered lotus by solid-phase microextraction	Choi (2017)
	Volatile aroma compounds and their characteristics of Labiatae by solid-phase microextraction (SPME)	Song et al. (2002)
	Comparison of volatile flavor compounds of Artemisia annua L. extracted by simultaneous steam distillation extraction and solid-phase micro extraction	Hong et al. (2018)
	Studies on the effect of low winter temperatures and harvest times on the volatile aroma compounds in green teas	Ryu et al. (2012)

Table 2
Previous studies on the aroma-volatile components of vegetable proteins.

Samples	Experimental items	Title	References
Soybean	Volatile compounds	Identification of volatile compounds in soybean at various developmental stages using solid phase microextraction	Boué et al. (2003)
Soy protein isolate	Volatile compounds	Volatile aroma components of soybean protein isolate and acid-hydrolysed vegetable protein	Solina et al. (2005)
Soy protein	Volatile compounds	Volatile components of an unflavored textured soybean protein	Ames and Macleod (1984)
Soybean protein	Volatile compounds	Insights into formation, detection, and removal of the beany flavor in soybean protein	Wang et al. (2021)
Pea, soybean	Volatile compounds	Key volatile off-flavor compounds in peas (Pisum sativum L.) and their relations with the endogenous precursors and enzymes using soybean (Glycine max) as a reference	Zhang et al. (2020)
Soybean, allergen-free pea, brown rice protein	Volatile compounds	A rapid gas-chromatography/mass-spectrometry technique for determining odor activity values of volatile compounds in plant proteins: soybean and allergen-free pea and brown rice protein	Singh et al. (2021)
Wheat, soybean, rice, pea protein	Functional properties (protein solubility, water absorption, SDS-PAGE)	Comparison of wheat, soybean, rice, and pea protein properties for effective applications in food products	Zhao et al. (2020)

Blending also harmonizes the flavors of each ingredient, softening undesirable notes and producing a richer, more balanced taste. The functional properties of these proteins—such as water retention, elasticity, and binding capacity—enhance the stability and quality of the final product (Imran and Liyan, 2023). This strategic blending optimizes the nutrition, texture, and flavor of TVP, meeting diverse consumer preferences and offering a versatile, appealing plant-based alternative (Schneider and Lacampagne, 2000; Park, 2022). Therefore, it must be strategically blended with other plant proteins, such as rice, wheat, and pea proteins, or replaced with alternative protein sources. (Sanchez-Monge et al., 2004; Yun et al., 2021).

Flavor is a critical factor in consumer preference, and replicating the flavor of meat is essential to bridging the sensory gap between plant-based substitutes and processed meat products. Plant-based meat often falls short in flavor and texture compared to conventional meat, highlighting the necessity for technological advancements (You et al., 2020). Legumes, a common protein source, have inherent off-flavors due to compounds like bitter phenols and saponins, contributing to sensory deficiencies in plant-based substitutes (Roland et al., 2017).

To address off-flavors in soybeans, widely used in plant-based meat, strategies include breeding lipoxygenase-deficient soybeans, enzyme inactivation during processing, and masking flavors with spices or seasonings (Yong et al., 2021; Kim et al., 2009a). However, compared to the significant body of research focused on improving the texture of plant-based meat substitutes, efforts to enhance their flavor profiles remain limited. Despite technological advancements that have significantly enhanced the texture and appearance of plant-based meat alternatives, research focusing on flavor and aroma improvements remains sparse. Bridging the sensory gap between plant-based proteins and traditional meat requires targeted research emphasis on flavor and aroma optimization. This gap underscores a critical opportunity for innovation in plant-based food development.

Among the various methods available for flavor analysis, headspace solid-phase microextraction (HS-SPME) is particularly effective for collecting volatile gaseous compounds from the headspace of a vial containing a solid or liquid sample. This technique, which uses a polymer phase to adsorb compounds onto silica fibers, is highly efficient, reduces sample pretreatment time, eliminates the need for solvents, and enables the straightforward, cost-effective analysis of organic components. Owing to these advantages, HS-SPME is widely used to analyze aroma compounds in various food products, including meat, dairy, tea, coffee, and wine. For plant-based meat substitutes, it plays a key role in identifying and addressing the inherent odors present in ingredients like soybeans. By analyzing the volatile compounds that contribute to the characteristic soybean aroma, HS-SPME can help identify off-flavors and provide insights into minimizing or eliminating these odors. As a result, HS-SPME becomes an essential tool in improving the sensory profile of plant-based meat alternatives, facilitating the development of products that are more appealing to consumers while retaining the nutritional benefits of plant-based ingredients. Table 1 outlines various studies that have investigated the aromatic components of various foods, showcasing how HS-SPME has been employed to optimize flavor profiles in alcohols, coffee and tea products (Jo et al., 2013; Lee, 2014; Shin and Lee, 2015; Kim and Lee, 2019; Kim et al., 2009b; Lee and Kim, 2023; Min et al., 2015; Her, 2021; Lee et al., 2011; Park et al., 2000; Choi, 2017; Song et al., 2002; Hong et al., 2018; Ryu et al., 2012).

Table 3
Sample code and country of origin of vegetable proteins used in this study.

Sample code	Country of origin	Ingredients	Food type
Rice-1	Spain	Rice protein 100%	Processed grain products
Rice-2	Vietnam	Rice protein 100%	Processed grain products
Wheat-1	France	Wheat gluten 100%	Processed grain products
Wheat-2	China	Wheat gluten 100%	Processed grain products
Soy-1	China	Isolated soybean protein 100%	Processed legumes products
Soy-2	Singapore	Isolated soybean protein 100%	Processed legumes products
Soy-3	United States	Isolated soybean protein 99.7%, Lecithin 0.3%	Processed legumes products
Soy-4	China	Isolated soybean protein 100%	Processed legumes products
Pea-1	Spain	Pea protein 100%	Processed legumes products
Pea-2	Germany	Pea protein 100%	Processed legumes products
Pea-3	Germany	Pea protein 100%	Processed legumes products
Pea-4	France	Pea protein 100%	Processed legumes products

Among the studies listed in Table 1, several have specifically analyzed the aroma profiles of vegetable proteins, including research on the aroma composition of soybeans (Boué et al., 2003), off-flavor analysis of isolated soybean protein (Ames and Macleod, 1984), a comparison of aroma composition following soy protein hydrolysis (Solina et al., 2005), and the aroma profiles of soybean, pea, and brown rice proteins (Singh et al., 2021). In addition to analyzing aroma compounds, various methods have been investigated to remove off-flavors and their causative agents. Studies on the structural characteristics of other plant proteins are also summarized in Table 2 (Boué et al., 2003; Ames and Macleod, 1984; Solina et al., 2005; Singh et al., 2021; Wang et al., 2021; Zhang et al., 2020; Zhao et al., 2020).

However, the literature on factors influencing flavor is largely confined to legumes and lacks comprehensive data, particularly studies comparing the flavor characteristics of various vegetable proteins.

In this study, we conducted a foundational investigation aimed at enhancing the flavor and sensory quality of plant-based meat substitute products. We compared the overall aroma profiles of textured vegetable proteins obtained from rice, wheat, soybean, and pea proteins, which are commonly used as vegetable protein sources. The goal of this study was to identify aroma components that serve as indicators of off-flavors and to establish these findings as foundational data for application in the development of plant-based meat substitutes.

2. Materials and methods

2.1. Chemicals and reagents

In this study, saturated alkanes standards C7–C30 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Eight indicator standard materials, including 3-methylbutanal, hexanal, benzaldehyde, nonanal, (E)-2-nonenal, 1-octen-3-ol, 2-pentylfuran, and 3-octen-2-one, were also obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical and chromatographic purity.

2.2. Materials

Two types of commercially available rice protein, two types of wheat gluten, four types of soybean protein, and four types of pea protein were selected for this study. Detailed sample information is provided in Table 3.

2.3. Extrusion of textured vegetable protein

The experiment utilized an intermeshing twin-screw extruder (Process-11, Thermo Fisher Scientific, Inc., Dreieich, Germany) equipped

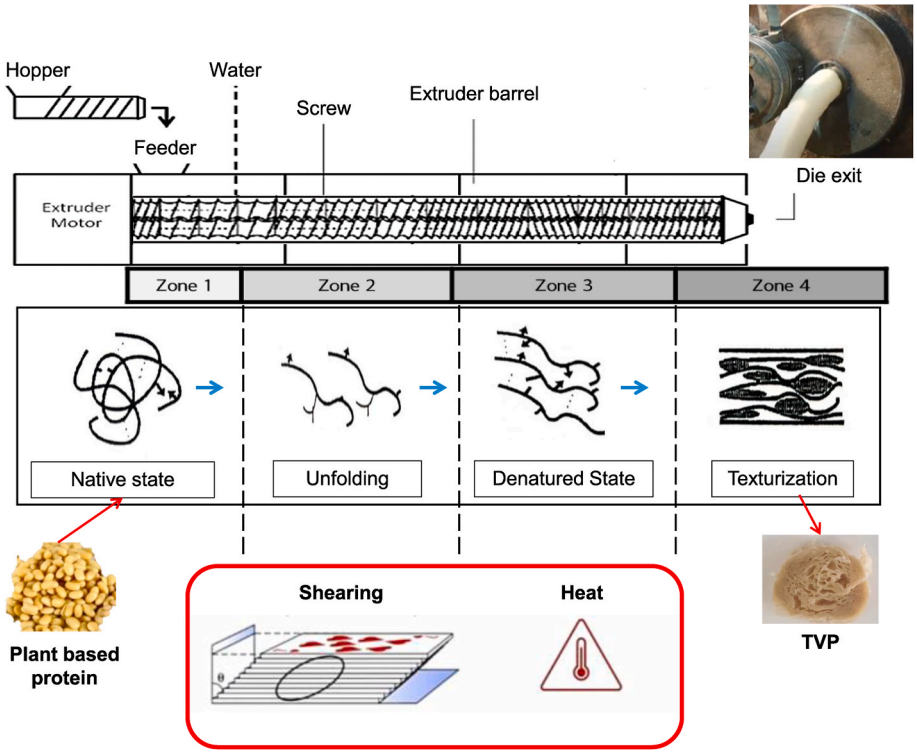

Fig. 1. Extrusion process of textured vegetable proteins.

Table 4
Operating conditions for detection of the aroma compounds of plant proteins.

Parameter	Condition
Instrument	Agilent 8890 GC and Agilent 7010B GC/TQ instruments GERSTEL gas syringe-type headspace autosampler MPS Robotic pro
SPME condition	Preconditioning 40 °C, 10 min, 500 rpm Extraction 50 °C, 30 min Desorption 250 °C, 5 min
Column	DB-WAX 60 m × 0.25 mm × 0.25 μm
Oven temp.	40 °C (5 min) > 3 °C/min to 230 °C > 230 °C (7 min)
MS interface temp.	150 °C
MS mode	Scanned from 40 to 500 m/z

with a cooling die attached to the end of a barrel to manufacture the extruded products. The extrusion conditions were a barrel temperature of 190 °C and a screw rotation speed of 250 rpm. Water was injected at a rate of 9 rpm using a metering pump (BT101S Peristaltic Pump Drive, Lead Fluid Technology Co., Baoding, China), and the raw material input was adjusted to 5 g/min. Fig. 1 shows the detailed molding process.

2.4. Sample preparation and HS-SPME extraction conditions

Headspace solid-phase microextraction (HS-SPME) was employed, followed by gas chromatography-mass spectrometry (GC-MS), to extract and analyze the volatile aromatic compounds from the plant protein samples. For the analysis, 1 g of each sample was mixed with 7 mL of distilled water, transferred to a headspace vial, and equilibrated by heating and shaking at 500 rpm for 10 min at 40 °C. A 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (548653-U, Supelco, PA, USA) was inserted into the headspace to adsorb the volatile compounds. The extraction was performed for 30 min at 50 °C. Following extraction, the volatiles were desorbed at 250 °C for 5 min. After each injection, the SPME fiber was conditioned at 250 °C under a helium flow for 10 min. The distilled water used in the sample preparation had a resistivity of 18.2 MΩ and was HPLC-grade (7732-18-5, Honeywell Burdick & Jackson, MI, USA).

2.5. GC-MS analysis method

GC-MS analysis was conducted to screen the aroma components using an Agilent 8890 GC and Agilent 7010B GC/TQ system (Agilent Technologies, CA, USA) equipped with a gas syringe-type headspace autosampler, MPS Robotic Pro (GERSTEL, MD, USA). An SPME-exclusive injector liner (2637505; Supelco, MO, USA) was employed for the splitless injection method. The GC column used was a DB-WAX (60 m × 0.25 mm × 0.25 μm) obtained from Agilent. The DB-WAX capillary column was operated under the following conditions: the oven temperature was initially set at 40 °C for 5 min, followed by an increase to 230 °C at a rate of 3 °C/min, and held at 230 °C for 7 min. The Agilent 7010B GC/TQ was operated in electron ionization mode at 70 eV, with the ion source temperature set at 150 °C and scanning from 40 to 500 m/z. Instrumental analysis conditions previously described in the literature (Singh et al., 2021; Chang et al., 2019) were followed, as summarized in Table 4. Saturated alkane standards C7–C30 were loaded into the injection port of the GC-MS system under identical chromatographic conditions to determine retention indices (RI). The compound-to-indicator standard peak area ratio determined the relative compound levels. GC-MS analyses were performed in triplicate.

2.6. Data analysis

Data were collected using Agilent MassHunter software (standard MSD version v10.2.489), and the recovered volatile compounds were screened against the National Institute of Standards and Technology (NIST20 v. 02) library (Palisade Corp., Newfield, NY, USA). A heat map was generated using Agilent's Mass Profiler Professional (MPP v15.1) software.

3. Results and discussion

3.1. Combined aroma profiling of rice, wheat, soybean, and pea proteins in TVP

Comprehensive fragrance profiling was conducted on rice, wheat, soybean, and pea proteins in TVP samples, identifying 16 alcohol compounds across 12 samples. 1-pentanol, 1-hexanol, benzyl alcohols, and 1-octen-3-ol were present in all pea protein samples, contributing to fermented, herbal, and earthy aromas. Additionally, 1-octen-3-ol, a compound commonly associated with grains, was detected in all samples, imparting specific off-flavor notes. 1-heptanol and 1-octanol were found exclusively in rice and pea proteins, with higher concentrations in rice proteins, adding unique aroma profiles. In wheat TVP samples, alcohols such as 1-butanol, 2-butanol, 2-heptanol, (E)-2-hepten-1-ol, and (E)-2-octen-1-ol provided distinct aromatic contributions. Similarly, 1-penten-3-ol and (Z)-2-hexen-1-ol, detected only in pea TVP samples, along with 2-methoxy-4-vinylphenol, 2-ethyl-1-hexanol, and 2-phenoxyethanol, found exclusively in rice TVP, further enhanced the aroma diversity among samples. This analysis demonstrates that these alcohol compounds, present in varying concentrations also distinctly shape the fragrance profiles of the TVP samples. The distribution and aroma contributions are summarized in Table 5.

Twenty-two aldehydes were identified across the 12 TVP samples, each contributing distinct aroma characteristics. Among these, 3-Methylbutanal, hexanal, benzaldehyde, 4-ethyl-2,4-benzaldehyde, and styrene exhibited aldehydic, green, fruity, and balsamic aromas. Among these, 3-methylbutanal, hexanal, nonanal, and benzaldehyde impart off-flavors commonly associated with grains. Pentanal, heptanal, octanal, (E)-2-nonanal, and nonanal, characterized by winey, green, aldehyde, and fatty aroma characteristics, were detected in rice, wheat, and soybean proteins but were absent in pea proteins. (E)-2-Nonanal and nonanal also contribute to off-flavors, similar to 3-methylbutanal, hexanal, and benzaldehyde. Butanal and 2-methylbutanal, with cocoa-like aromas, were present in all TVP samples but at higher concentrations in rice and pea TVP samples. Unique to rice and wheat TVP were 2-butyl-octenal, (E)-cinnamaldehydes, (E,E)-2,4-decadienal, and 2-methylpropanal, while (E)-2-decenal was found exclusively in rice TVP. Conversely, (E)-2-hexenal, 2-methyl-2-butenal, and 2-methyl-2-pentenal were identified only in soybean and pea TVP samples. These findings highlight distinct differences in aldehyde profiles among rice and wheat TVP samples compared to soybean and pea TVP samples. Table 6 summarizes the identified aldehydes and their contributions to the aroma profiles of the TVP samples.

Eleven ketones, three furans, and three sulfides were identified across the 12 TVP samples, each contributing specific aroma characteristics. Among the ketones, 3-octen-2-one (earthy and creamy aroma) and 2-heptanone (cheesy aroma) were more concentrated in rice and wheat TVP samples but occurred in lower concentrations in soybean and pea TVP samples. 6-Methyl-5-hepten-2-one, known for its citrus and green aromas, was detected at similar levels in soybean and rice TVP samples. Ketones such as 2-decanone, 2-octanone, and 2-nonanone, and (E,E)-3,5-octadien-2-one, were present in all TVP samples, while (E,E)-3,5-octadien-2-one was exclusive to rice and wheat TVP, and 3-octanone was found only in soybean and pea proteins. For furans, 2-pentylfuran, associated with a green aroma and an unusual soybean taste, was abundant in wheat and pea TVP samples but less prevalent in rice and soybean samples. 2-n-butylfuran, characterized by a bright odor, was detected in all samples except wheat TVP. Additionally, 5-hydroxymethylfurfural was identified exclusively in soybean TVP samples. Among sulfur compounds, methanethiol was detected only in soybean and pea TVP samples. Dimethyl disulfide, absent in wheat TVP, was

Table 5
Aroma profiles and concentrations of alcohols in rice-, wheat-, soybean-, and pea-textured vegetable protein samples.

No.	Retention index	Compound name	CAS#	Textured vegetable protein Sample Conc. (μg/L)											
				Rice-1	Rice-2	Wheat-1	Wheat-2	Soybean-1	Soybean-2	Soybean-3	Soybean-4	Pea-1	Pea-2	Pea-3	Pea-4
1	765	1-Octen-3-ol	3391-86-4	1883.61 ± 0.67	1263.25 ± 0.32	764.11 ± 0.41	757.86 ± 0.52	318.63 ± 0.68	354.55 ± 0.44	351.83 ± 0.51	341.57 ± 0.44	196.11 ± 0.89	148.39 ± 0.32	147.71 ± 0.07	185.40 ± 0.89
2	868	1-Pentanol	71-41-0	520.81 ± 0.97	504.58 ± 0.48	358.98 ± 0.7	334.99 ± 0.68	144.40 ± 0.87	104.64 ± 0.20	126.21 ± 0.82	111.22 ± 0.30	327.22 ± 0.12	310.63 ± 0.24	331.76 ± 0.88	306.42 ± 0.74
3	980	1-Hexanol	111-27-3	320.27 ± 0.08	378.62 ± 0.18	992.07 ± 0.29	934.1 ± 0.16	120.33 ± 0.5	120.96 ± 0.29	250.39 ± 0.47	233.33 ± 0.59	856.95 ± 0.97	862.09 ± 0.74	834.7 ± 0.32	886.15 ± 0.27
4	970	Benzyl alcohols	100-51-6	36.57 ± 0.94	35.61 ± 0.48	1.73 ± 0.83	1.57 ± 0.29	5.73 ± 0.74	4.44 ± 0.47	3.98 ± 0.52	4.08 ± 0.04	14.75 ± 0.75	13.38 ± 0.88	15.29 ± 0.11	11.88 ± 0.25
5	1070	1-Heptanol	111-70-6	220.17 ± 0.71	151.21 ± 0.68	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	16.02 ± 0.67	19.58 ± 0.73	26.64 ± 0.46	20.67 ± 0.47
6	598	1-Octanol	111-87-5	155.10 ± 0.49	159.42 ± 0.25	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5.73 ± 0.90	6.74 ± 0.13	7.11 ± 0.87	6.44 ± 0.67
7	659	2-Butanol	78-92-2	N.D.	N.D.	0.87 ± 0.61	1.26 ± 0.21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
8	900	1-Butanol	71-36-3	N.D.	N.D.	0.87 ± 0.95	1.27 ± 0.77	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
9	978	2-Heptanol	543-49-7	N.D.	N.D.	50.57 ± 0.91	49.72 ± 0.07	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
10	1067	(*E*)-2-Hepten-1-ol	33467-76-4	N.D.	N.D.	34.49 ± 0.17	30.47 ± 0.29	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11	683	(*E*)-2-Octen-1-ol	18409-17-1	N.D.	N.D.	24.74 ± 0.64	25.19 ± 0.61	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
12	868	1-Penten-3-ol	616-25-1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	149.30 ± 0.60	150.74 ± 0.86	157.48 ± 0.05	160.34 ± 0.15
13	736	(*Z*)-2-Hexen-1-ol	928-94-9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5.73 ± 0.90	4.77 ± 0.24	5.34 ± 0.89	3.65 ± 0.35
14	1036	2-Methoxy-4-vinylphenol	7786-61-0	25.53 ± 0.34	24.47 ± 0.84	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
15	1316	2-ethyl-1-hexanol	104-76-7	86.73 ± 0.16	85.01 ± 0.03	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
16	1030	2-phenoxyethanol	122-99-6	79.53 ± 0.31	64.58 ± 0.86	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D., not detected.
Data are presented as mean values ± standard deviation (SD) obtained from three independent analyses (n = 3).

Table 6
Aroma profiles and concentrations of aldehydes in rice-, wheat-, soybean-, and pea-textured vegetable protein samples.

No.	Retention index	Compound name	CAS#	Textured vegetable protein Sample Conc. (μg/L)											
				Rice-1	Rice-2	Wheat-1	Wheat-2	Soybean-1	Soybean-2	Soybean-3	Soybean-4	Pea-1	Pea-2	Pea-3	Pea-4
1	801	Hexanal	66-25-1	1777.57 ± 0.83	2251.15 ± 0.64	108.10 ± 0.79	677.93 ± 0.78	1446.35 ± 0.05	1468.71 ± 0.51	1299.23 ± 0.33	877.27 ± 0.57	719.61 ± 0.31	890.95 ± 0.82	153.91 ± 0.20	135.32 ± 0.67
2	652	2-Methylbutanal	590-86-3	92.51 ± 0.54	99.42 ± 0.16	77.29 ± 0.38	15.57 ± 0.51	166.91 ± 0.65	125.65 ± 0.98	174.47 ± 0.47	179.13 ± 0.09	25.18 ± 0.80	23.53 ± 0.03	22.69 ± 0.79	24.57 ± 0.71
3	962	Benzaldehydes	100-52-7	1247.76 ± 0.46	1459.62 ± 0.54	587.50 ± 0.66	101.86 ± 0.39	328.38 ± 0.41	981.19 ± 0.62	592.68 ± 0.89	867.34 ± 0.12	238 ± 0.46	97.72 ± 0.41	196.08 ± 0.50	181.71 ± 0.60
4	1104	Nonanal	124-19-6	309.27 ± 0.07	273.25 ± 0.23	160.21 ± 0.12	11.47 ± 0.07	15.57 ± 0.26	16.79 ± 0.78	17.49 ± 0.80	19.92 ± 0.84	N.D.	N.D.	N.D.	N.D.
5	1162	(*E*)-2-Nonenal	18829-56-6	49.37 ± 0.65	49.72 ± 0.09	103.98 ± 0.71	105.83 ± 0.45	4.59 ± 0.31	4.05 ± 0.31	5.19 ± 0.70	4.98 ± 0.69	N.D.	N.D.	N.D.	N.D.
6	593	Butanal	123-72-8	46.05 ± 0.34	23.88 ± 0.62	5.21 ± 1.00	4.73 ± 0.67	3.95 ± 0.30	4.76 ± 0.83	24.33 ± 0.05	13.88 ± 0.09	37.75 ± 0.31	24.00 ± 0.79	30.47 ± 0.81	27.54 ± 0.74
7	662	2-Methylbutanal	96-17-3	94.21 ± 0.31	96.75 ± 0.96	43.71 ± 0.84	40.79 ± 0.91	15.75 ± 0.20	9.88 ± 0.55	14.14 ± 0.82	13.06 ± 0.37	29.47 ± 0.51	12.02 ± 0.68	7.27 ± 0.99	18.2 ± 0.41
8	958	(*E*)-2-Heptenal	18829-55-5	419.09 ± 0.31	495.86 ± 0.53	10.97 ± 0.41	10.59 ± 0.57	31.07 ± 0.66	29.58 ± 0.31	76.65 ± 0.68	35.88 ± 0.93	65.09 ± 0.53	61.43 ± 0.71	59.48 ± 0.68	56.18 ± 0.05
9	1060	(*E*)-2-Octenal	2548-87-0	209.27 ± 0.75	319.84 ± 0.23	7.80 ± 0.35	6.18 ± 0.97	22.36 ± 0.01	23.80 ± 0.65	44.13 ± 0.06	32.15 ± 0.87	10.49 ± 0.61	10.75 ± 0.96	10.89 ± 0.79	10.83 ± 0.88
10	1180	4-Ethyl-benzaldehydes	4748-78-1	100.23 ± 0.84	97.12 ± 0.58	12.96 ± 0.33	11.58 ± 0.48	18.93 ± 0.56	15.51 ± 0.74	19.98 ± 0.15	17.10 ± 0.53	19.72 ± 0.16	7.78 ± 0.53	7.29 ± 0.89	3.48 ± 0.34
11	893	Styrene	100-42-5	309.75 ± 0.90	327.23 ± 0.24	63.33 ± 0.06	67.11 ± 0.43	10.94 ± 0.43	19.44 ± 0.12	12.95 ± 0.93	9.47 ± 0.63	19.99 ± 0.35	10.90 ± 0.64	25.64 ± 0.72	10.35 ± 0.80
12	700	Pentanal	110-62-3	1004.52 ± 0.36	993.49 ± 0.83	250.54 ± 0.74	250.36 ± 0.64	468.12 ± 0.78	461.76 ± 0.35	568.16 ± 0.66	455.31 ± 0.59	N.D.	N.D.	N.D.	N.D.
13	901	Heptanal	111-71-7	747.10 ± 0.09	685.29 ± 0.69	494.37 ± 0.94	475.71 ± 0.94	124.54 ± 1.47	155.07 ± 0.44	140.25 ± 0.51	187.18 ± 0.01	N.D.	N.D.	N.D.	N.D.
14	1003	Octanal	124-13-0	324.17 ± 0.49	317.88 ± 0.23	16.91 ± 0.12	10.74 ± 0.81	50.72 ± 0.96	41.95 ± 0.09	41.49 ± 0.80	46.54 ± 0.09	N.D.	N.D.	N.D.	N.D.
15	1378	2-Butyl-2-octenal	13019-16-4	167.62 ± 0.88	273.39 ± 0.71	20.41 ± 0.36	21.20 ± 0.09	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
16	1270	(*E*)-Cinnamaldehydes	14371-10-9	54.81 ± 0.17	57.57 ± 0.98	32.55 ± 0.36	31.57 ± 0.85	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
17	1263	(*E*)-2-Decenal	3913-81-3	39.75 ± 0.65	34.61 ± 0.06	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
18	1317	(*E,E*)-2,4-Decadienal	25152-84-5	299.16 ± 0.67	226.36 ± 0.42	104.45 ± 0.50	109.81 ± 0.09	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
19	552	2-Methylpropanal	78-84-2	N.D.	N.D.	1106.63 ± 0.35	1074.53 ± 0.72	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
20	854	(*E*)-2-Hexenal	6728-26-3	N.D.	N.D.	N.D.	N.D.	1045.42 ± 0.46	1051.94 ± 0.21	1054.71 ± 0.57	1105.15 ± 0.13	20.57 ± 0.12	21.11 ± 0.63	23.59 ± 0.11	27.37 ± 0.13
21	746	2-Methy-2-butenal	1115-11-3	N.D.	N.D.	N.D.	N.D.	186.49 ± 0.95	200.19 ± 0.16	198.11 ± 0.24	153.41 ± 0.97	24.24 ± 0.19	25.37 ± 0.69	27.79 ± 0.38	30.14 ± 0.15
22	837	2-Methyl-2-pentenal	623-36-9	N.D.	N.D.	N.D.	N.D.	26.14 ± 0.86	28.56 ± 0.35	27.26 ± 0.05	26.26 ± 0.98	5.19 ± 0.47	5.79 ± 0.19	4.47 ± 0.77	4.59 ± 0.89

N.D., not detected.
Data are presented as mean values ± standard deviation (SD) obtained from three independent analyses (n = 3).

Table 7
Aroma profiles and concentrations of ketones, furans, and sulfur compounds in rice, wheat, soybean, and pea-textured vegetable protein samples.

No.	Retention index	Compound name	CAS#	Textured vegetable protein Sample Conc. (μg/L)											
				Rice-1	Rice-2	Wheat-1	Wheat-2	Soybean-1	Soybean-2	Soybean-3	Soybean-4	Pea-1	Pea-2	Pea-3	Pea-4
1	1040	3-Octen-2-one	1669-44-9	630.50 ± 0.52	673.12 ± 0.85	313.96 ± 0.16	346.81 ± 0.54	47.74 ± 0.22	46.90 ± 0.92	57.89 ± 0.06	40.46 ± 0.30	103.64 ± 0.47	118.62 ± 0.16	105.19 ± 0.61	105.56 ± 0.04
2	891	2-Heptanone	110-43-0	1473.57 ± 0.91	1458.52 ± 0.98	659.91 ± 0.55	678.91 ± 0.10	448.16 ± 0.97	365.69 ± 0.89	401.47 ± 0.16	443.39 ± 0.10	948.16 ± 0.22	977.27 ± 0.79	978.94 ± 0.88	913.16 ± 0.39
3	1193	2-Decanone	693-54-9	730.56 ± 0.70	781.16 ± 0.65	204.47 ± 0.52	207.79 ± 0.30	28.50 ± 0.83	29.57 ± 0.05	28.45 ± 0.35	30.19 ± 0.35	40.18 ± 0.87	54.54 ± 0.72	32.69 ± 0.11	49.65 ± 0.50
4	986	6-Methyl-5-hepten-2-one	110-93-0	353.64 ± 0.80	258.08 ± 0.95	85.27 ± 0.25	84.32 ± 0.09	196.48 ± 0.51	208.60 ± 0.66	268.36 ± 0.77	207.63 ± 0.45	20.76 ± 0.87	19.49 ± 0.23	19.86 ± 0.36	18.19 ± 0.74
5	991	2-Octanone	111-13-7	592.29 ± 0.06	537.37 ± 0.51	312.90 ± 0.87	312.01 ± 0.25	183.01 ± 0.90	207.28 ± 0.76	209.81 ± 0.38	138.77 ± 0.78	267.51 ± 0.91	252.98 ± 0.97	202.93 ± 0.47	245.91 ± 0.63
6	1092	2-Nonanone	821-55-6	90.68 ± 0.78	89.95 ± 0.47	27.81 ± 0.16	30.49 ± 0.32	44.76 ± 0.86	50.74 ± 0.48	51.73 ± 0.64	47.82 ± 0.15	392.24 ± 0.44	345.73 ± 0.07	382.19 ± 0.06	389.54 ± 0.68
7	1073	(*E,E*)-3,5-Octadien-2-one	30086-02-3	405.69 ± 0.90	395.55 ± 0.46	175.18 ± 0.27	183.67 ± 0.46	227.73 ± 0.02	135.76 ± 0.56	244.59 ± 0.63	162.7 ± 0.86	127.94 ± 0.78	138.06 ± 0.29	118.36 ± 0.05	163.85 ± 0.80
8	1091	3,5-Octadien-2-one	38284-27-4	367.61 ± 0.77	386.54 ± 0.99	154.41 ± 0.54	177.46 ± 0.60	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11	986	3-Octanone	106-68-3	N.D.	N.D.	N.D.	N.D.	7.89 ± 0.45	6.75 ± 0.07	6.36 ± 0.22	8.76 ± 0.23	18.78 ± 0.49	19.62 ± 0.18	20.67 ± 0.69	25.49 ± 0.23
12	993	2-Pentylfuran	3777-69-3	589.16 ± 0.39	587.63 ± 0.24	1063.83 ± 0.15	1200.01 ± 0.61	846.16 ± 0.25	845.93 ± 0.21	761.91 ± 0.24	801.64 ± 0.01	1004.19 ± 0.74	925.15 ± 0.52	998.81 ± 0.56	934.8 ± 0.01
13	893	2-n-Butyl furan	4466-24-4	983.72 ± 0.53	791.85 ± 0.33	N.D.	N.D.	343.25 ± 0.24	353.18 ± 1.00	374.36 ± 0.33	323.28 ± 0.69	30.86 ± 0.08	139.79 ± 0.41	99.19 ± 0.99	50.75 ± 0.22
14	1232	5-Hydroxymethylfurfural	67-47-0	N.D.	N.D.	N.D.	N.D.	30.63 ± 0.40	30.91 ± 0.33	27.41 ± 0.52	31.79 ± 0.99	N.D.	N.D.	N.D.	N.D.
15	401	Methanethiol	74-93-1	N.D.	N.D.	N.D.	N.D.	7.58 ± 0.38	6.03 ± 0.84	8.73 ± 0.59	5.57 ± 1.12	3.75 ± 0.82	3.72 ± 0.49	7.57 ± 0.59	5.07 ± 0.84
16	746	Dimethyl disulfide	624-92-0	866.31 ± 0.69	861.65 ± 0.26	N.D.	N.D.	127.41 ± 0.44	112.74 ± 0.98	93.28 ± 0.16	263.77 ± 0.77	37.79 ± 0.07	40.71 ± 0.37	45.19 ± 0.92	39.16 ± 0.05
17	971	Dimethyl trisulfide	3658-80-8	673.49 ± 0.33	697.67 ± 0.14	N.D.	N.D.	111.22 ± 0.25	138.68 ± 0.16	175.30 ± 0.69	103.10 ± 0.19	N.D.	N.D.	N.D.	N.D.

N.D., not detected.
Data are presented as mean values ± standard deviation (SD) obtained from three independent analyses (n = 3).

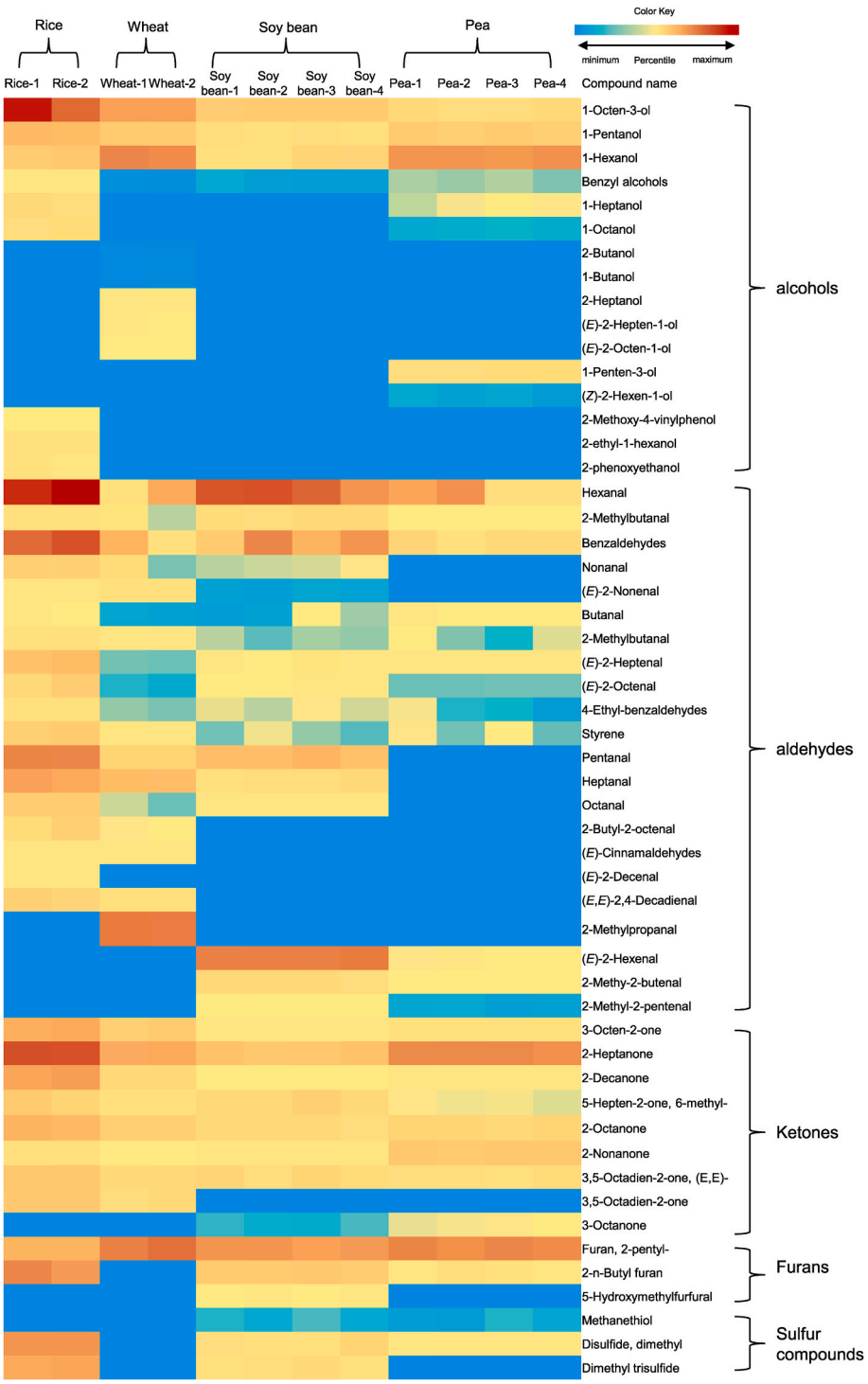

Fig. 2. Heat map of the individual samples of rice-, wheat-, soybean-, and pea-textured vegetable proteins.

found in all other samples, with rice TVP exhibiting the highest levels. Dimethyl trisulfide, present only in rice and soybean TVP samples, showed the highest concentrations in rice TVP. Table 7 summarizes the distribution and aroma profiles of the identified ketone, furan, and sulfur compounds.

Agilent Mass Profiler Professional (MPP) software was used to analyze all samples simultaneously. Fig. 2 presents a heat map comparing the detection status of all compounds in rice, wheat, soybean, and pea TVP samples. Detected compounds are shown in red, while undetected compounds are shown in blue. The heat map is displayed so that these trends can be viewed at a glance.

3.2. Comparison of the eight major aroma volatile markers

The volatile compounds associated with off-flavors in rice, wheat, soybean, and pea TVP include fatty aldehydes, fatty alcohols, fatty ketones, furans, furan derivatives, aromatic compounds, aromatic hydrocarbons, aldehydes, alkanes, alcohols, and ketones. The key indicator compounds identified were hexanal, nonanal, 2-nonenal, 3-methylbutanal, benzaldehyde, 1-octen-3-ol, 3-octen-2-one, and 2-pentylfuran,

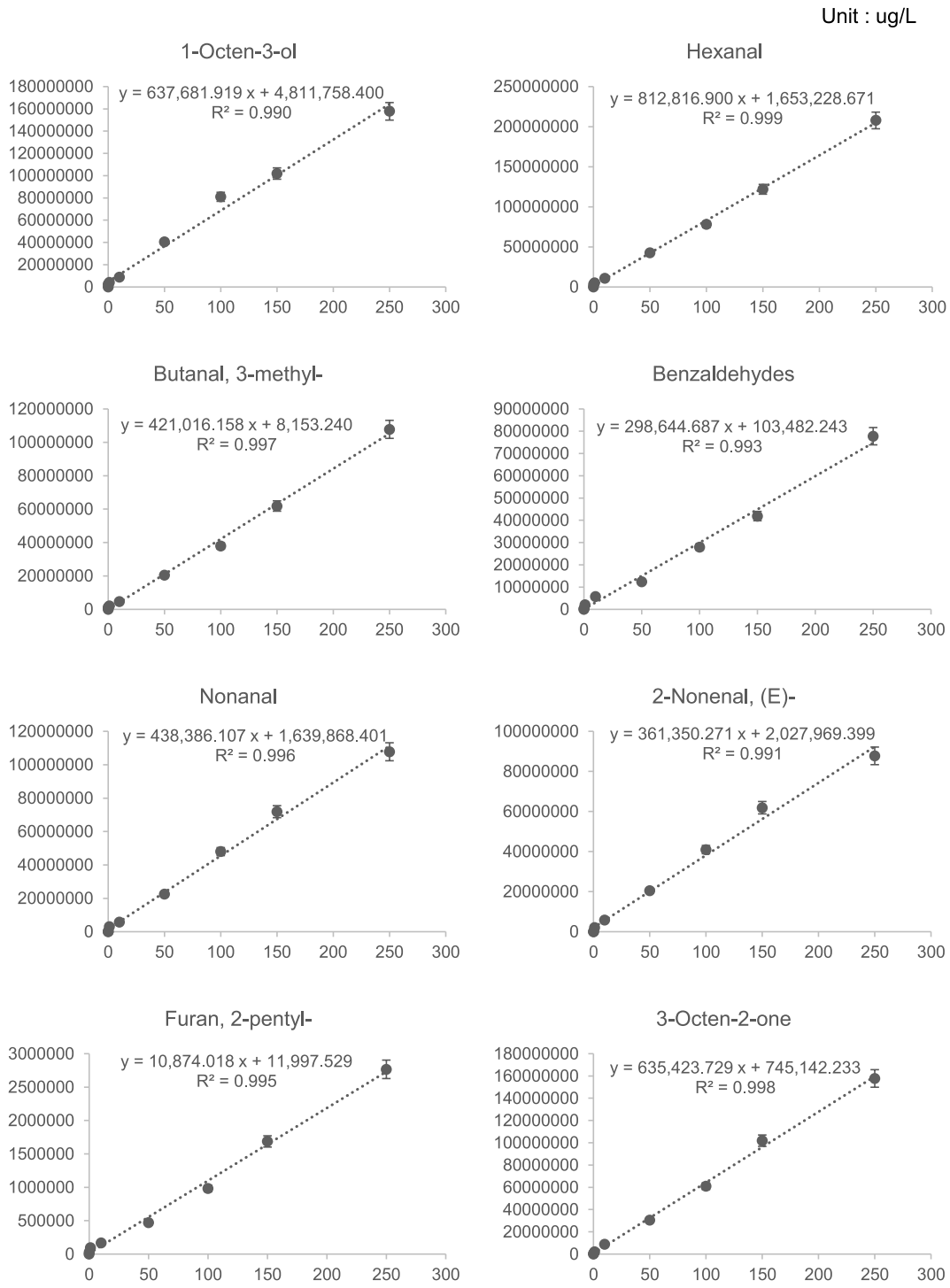

Fig. 3. Standard curve of eight major aroma volatile markers.

which are recognized as volatile marker compounds. Retention times and RI were determined for the eight major aroma volatile markers using standards, and a calibration curve was generated over the range of 0.1–300 μg/L. The R square values for all eight components were 0.99 or higher. Notably, the R square value of hexanal was 0.999 or higher. Each calibration curve is shown in Fig. 3.

Based on the calibration curves, the concentrations and deviations of the eight volatile markers are shown in Fig. 4.

1-Octen-3-ol, also known as mushroom alcohol, attracts insects and is present in human breath and sweat. It is produced by plants and fungi, including edible mushrooms and lemon balm (Wnuk et al., 1983; Wood et al., 2001). The concentration of 1-octen-3-ol was the highest in rice TVP samples (1573.43 μg/L), followed by wheat TVP (760.99 μg/L), soybean TVP (341.65 μg/L), and pea TVP (169.40 μg/L).

Hexanal, also known as caproaldehyde, is an alkyl aldehyde with a freshly cut grass aroma. It naturally contributes to the off-flavors and off-notes in soybeans and peas (Roland et al., 2017). The concentration of hexanal was the highest in rice TVP (2014.36 μg/L), followed by soybean TVP (1272.89 μg/L), pea TVP (449.65 μg/L), and wheat (393.02 μg/L). Interestingly, the high hexanal levels in rice suggest that this compound affects not only legumes but also rice.

3-Methylbutanal, an isovaleraldehyde from the aldehyde family, is

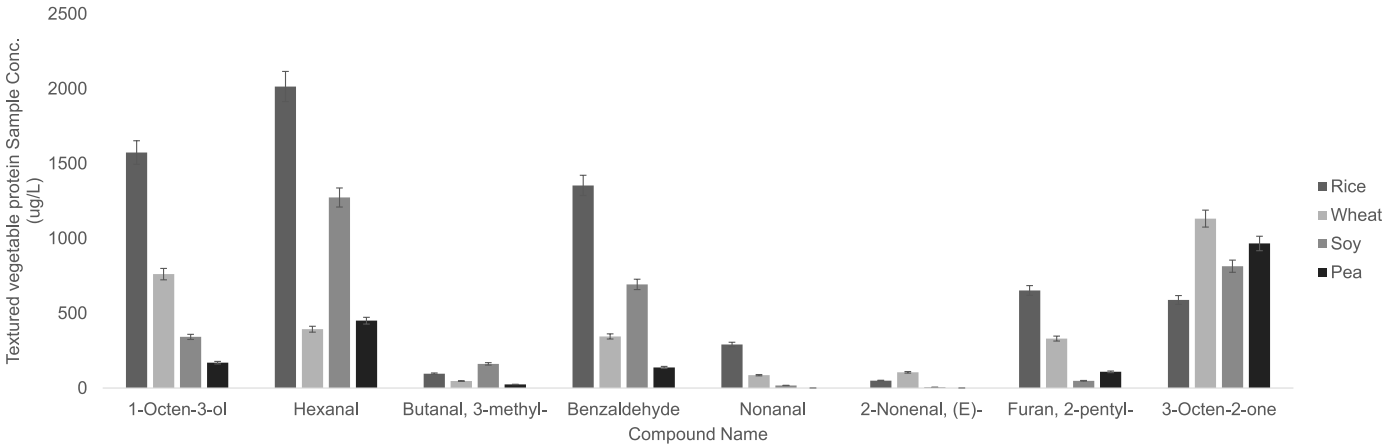

Fig. 4. Concentrations and standard deviations of the eight major aroma volatile markers.

derived from leucine and is commonly used as a flavoring agent in foods. It is typically described as having a malty flavor and is present in various foods such as cheese, coffee, chicken, fish, chocolate, olive oil, and tea (Cserháti and Forgács, 2003; Owuor, 2003). Due to its putrid aroma, it is also regarded as an indicator of off-flavor. The concentrations of butanal and 3-methylbutanal were compared in rice, wheat, soybean, and pea TVP samples. Soybean protein contained the highest concentration (161.54 μg/L), followed by rice (95.97 μg/L), wheat (46.43 μg/L), and pea (23.99 mg/L).

Benzaldehyde, the simplest aromatic aldehyde, contains a benzene ring with a formyl substituent and has a characteristic almond-like aroma (Adams et al., 2005). Amygdalin, found in almonds, apricots, apples, and cherry seeds, is enzymatically decomposed into benzaldehyde and glucose. It is widely used in flavoring cosmetics, e-cigarettes, and food products (Andersen, 2006). The concentration of benzaldehyde was the highest in rice TVP (1353.69 μg/L), followed by soybeans (692.40 μg/L), wheat (344.68 μg/L), and peas (137.63 μg/L).

Nonanal, also known as pelargonaldehyde, is a common ingredient in perfumes and natural oils but is also known for its aldehydic off-flavor, which can attract mosquitoes (Syed and Leal, 2009). The concentration of nonanal was the highest in rice (291.26 μg/L), followed by wheat (85.84 μg/L) and soybean TVP samples (17.44 μg/L). Nonanal was not detected in pea TVP.

(*E*)-2-Nonanal, an unsaturated aldehyde, is an important aroma compound in aged beer and buckwheat (Santos et al., 2003). It has a fatty, cucumber-like smell and is also associated with body odor in aging (Haze et al., 2001). The concentration of (*E*)-2-nonenal was the highest in wheat TVP (104.91 μg/L), followed by rice (49.54 μg/L) and soybean (4.70 μg/L), and was not detected in pea TVP.

2-Pentylfuran, a component of the furan series, is characterized by its unique fruity scent. Its concentration was the highest in rice (651.81 μg/L), followed by wheat (330.39 μg/L), pea (108.25 μg/L), and soybean (48.25 μg/L).

3-Octen-2-one, a ketone with an earthy, fermented aroma, was detected in wheat (1131.92 μg/L), pea (965.74 μg/L), soybean (813.91 μg/L), and rice TVP (588.40 μg/L). The impact of off-flavor indicator components was relatively minor in pea TVP samples, whereas the effects of hexanal and benzaldehyde were significant in rice protein, comparable to their impact on soybean and wheat TVPs.

4. Conclusions

To compare the volatile aroma properties of TVP, isolated protein samples from rice, wheat, soybean, and peas were selected. The volatile components of the samples were profiled, compared, and verified. Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS), a specialized technique for aroma analysis, was utilized to obtain the retention indices and mass spectra of all components in the samples. The scent components were categorized into chemical families based on their functional groups and profiled by identifying the characteristic distribution of the chemical components in each sample. Additionally, the relative content of each sample was visualized on a heat map rendered in color. Eight components (hexanal, nonanal, 2-nonenal, 3-methylbutanal, benzaldehyde, 1-octen-3-ol, 3-octen-2-one, and 2-pentylfuran) were selected as off-flavor markers based on the profiling results and previous studies. The aroma component profile of each TVP was characterized, providing valuable insights for optimizing conditions to reduce off-flavors in vegetable protein raw materials. Rice and wheat TVPs are grain-based TVPs. In this regard, the flavor component profiles and concentrations of major volatile markers indicated that 1-octen-3-ol, hexanal, and benzaldehyde were present at high concentrations in rice TVP, suggesting that they may affect its flavor and taste. In contrast, soybean TVP and pea TVP are legume-based, and they were lower than grain-based TVPs. In particular, pea TVP contained the lowest concentration of 3-octen-2-one, suggesting that it may have a lesser effect on its flavor and taste.

As the demand for plant-based proteins continues to grow, the comparative results of this study highlight the potential for controlling the flavor of TVPs by strategically selecting raw materials. In this study, we analyzed and verified the basic flavor components involved in TVP manufacturing through flavor profiling of the four main TVP raw materials: rice, wheat, soybeans, and peas. In future studies, we aim to investigate the effects of enzymatic, physical, and chemical methods for off-flavor reduction. We hope that this research will be useful for other studies. We believe that these findings could contribute to the development of next-generation vegetable proteins and help replicate meat flavors in TVPs.

CRediT authorship contribution statement

Geon-Woo Park: Conception and design of the study, acquisition of data, data analysis and/or interpretation, drafting of the manuscript and/or critical revision. **Kyung-Ho Park:** Conception and design of the study, data analysis and/or interpretation, drafting of the manuscript and/or critical revision. **Sang-Gu Kim:** Conception and design of the study. **Sang-Yun Lee:** Conception and design of the study, All authors have read and approved the published version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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