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Mehrsa Emkani, Karine Gourrat, Bonastre Oliete, Rémi Saurel. Identification of volatile and odor-active compounds in pea protein fractions obtained by a modified extraction method using fermentation. *Journal of Food Science*, 2024, 7, pp.4229-4249. 10.1111/1750-3841.17145 . hal-04616049

HAL Id: hal-04616049

<https://institut-agro-dijon.hal.science/hal-04616049v1>

Submitted on 18 Jun 2024

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ORIGINAL ARTICLE

Food Chemistry

Identification of volatile and odor-active compounds in pea protein fractions obtained by a modified extraction method using fermentation

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Funding information

French Agence Nationale de la Recherche, Grant/Award Number: ANR-19-CE21-0008-03; European Regional Development Fund, Grant/Award Number: BG0025913

Abstract: This study investigates the aromatic composition of pea albumin and globulin fractions obtained through either fermentation or conventional acidification using hydrochloric acid (control) toward the isoelectric point of pea globulins. Different lactic acid bacteria were used including *S. thermophilus* (ST), *L. plantarum* (LP), and their coculture (STLP). The volatile compounds were extracted by solvent-assisted flavor evaporation technique and quantified by gas chromatography–mass spectrometry (GC-MS). Odor-active compounds (OAC) were further characterized by gas chromatography–olfactometry (GC-O). In total, 96 volatile and 36 OACs were identified by GC-MS and GC-O, respectively. The results indicated that the protein fractions obtained by conventional acidification were mainly described by green notes for the presence of different volatile compounds such as hexanal. However, the samples obtained by fermentation had a lower content of these volatile compounds. Moreover, protein fractions obtained by coculture fermentation were described by volatile compounds associated with fruity, floral, and lactic notes.

KEYWORDS

Pea albumin and globulin, aroma compounds, gas chromatography–olfactometry, GC-MS, lactic acid bacteria

Practical Application: The insights from this study on pea protein aroma could find practical use in the food industry to enhance the sensory qualities of plant-based products. By utilizing fermentation methods and specific lactic acid bacteria combinations, manufacturers may produce pea protein with reduced undesirable green notes, offering consumers food options with improved flavors. This research may contribute to the development of plant-based foods that not only provide nutritional benefits but also meet consumer preferences for a more appealing taste profile.

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1 | INTRODUCTION

Despite its popularity, pea protein is associated with off-flavors that can hinder their application in food products. The off-flavors in peas are mainly described as green and beany, which can arise from various stages of production, such as harvest, storage, and processing (Roland et al., 2017). Off-flavors result from fatty acid oxidation, amino acid biodegradation, heat effects, carotenoid degradation, and extraction contaminations (Murray et al., 1976; Roland et al., 2017). Better controlling raw materials, extraction, and processing conditions can improve the aromatic profile of pea proteins. Lactic acid fermentation could be promising for reducing these off-flavors by intrinsic enzymatic activity and/or by producing new volatile compounds through the metabolic pathways (Ben-Harb et al., 2019; Fischer et al., 2022b; Schindler et al., 2011).

A recent study in our team represented an alternative extraction method based on alkaline solubilization/isoelectric precipitation. In this approach, the reduction of pH was obtained thanks to the production of lactic acid by specific starters during fermentation, which leads to the precipitation of nonsoluble fractions (globulins) and their separation from the soluble ones (albumins) (Emkani et al., 2021). They have also shown the albumin fraction obtained by this method had a higher content of total nitrogen compared with conventional acidification without damaging the structure of globulin fraction, which is used as pea protein isolate in industry. This was explained by the proteolytic activity of the lactic acid bacteria (LAB) leading to the production of small peptides and amino acids that were solubilized and recovered in the soluble albumin-rich fraction (Emkani et al., 2023). Considering the enzymatic activity of LAB during the metabolism of different nutrients and production of precursors of volatile compounds (such as different amino acids), which can positively improve the aromatic profile of legume proteins, the purpose of this study was to identify the volatile compounds of albumin and globulin fractions obtained without (control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP). Volatile compounds were isolated by the solvent-assisted flavor evaporation (SAFE) method as the method enables the maximum isolation of volatile compounds from the matrix. The isolated volatile compounds were subsequently characterized by gas chromatography–mass spectrometry (GC-MS). The identification of volatile compounds was completed by the detection of odor-active compounds (OAC) by gas chromatography–olfactometry (GC-O).

2 | MATERIALS AND METHODS

2.1 | Materials

All the materials were of analytical grade and supplied by Honeywell Fluka™ (Gillman, SA, Australia) or Thermo Fisher (Dardilly, France), unless the contrary was indicated. Pea seed (Assas cultivar) was provided by INRAE (UMR AgroEcologie, Dijon, France), as part of PEAVALEU project ANR-19-CE21-0008-03. Freeze-dried LAB, including *Streptococcus thermophilus* (102303T) (ST) and *Lactiplantibacillus plantarum* (CNRZ211) (LP), were purchased from the International Center for Microbial Resources–food Associated Bacteria (CIRM-BIA, Rennes, France). The selection of the bacteria was done according to a study performed by our group (Emkani et al. 2023).

2.2 | Methods

2.2.1 | Bacterial culture

The bacterial culture was performed according to Emkani et al. (2023). Briefly, freeze-dried bacteria were rehydrated in MRS medium for LP and M17 medium for ST and then incubated at their optimal temperature (37°C for LP, 43°C for ST) for 24 h. The cells were isolated on agar medium, and a single colony was incubated in 10 mL of liquid medium for 24 h. A culture was created by inoculating 10 mL of broth with 1 mL of preculture, stopped at stationary phase (around 12 h for LP, 7 h for ST). The cell suspension was centrifuged (Eppendorf® Centrifuge 5804/5804R, USA) (4000 × g, 20°C, 5 min), resuspended in 1 mL of fresh broth with 30% glycerol, and stored at –80°C.

For protein extraction, the contents were transferred to a sterile tube. Glycerol was removed by centrifugation (4000 × g, 5 min, 4°C), replaced with 1 mL of fresh broth, washed three times, and incubated in 10 mL of fresh broth medium for 24 h at optimal growth temperature. Monoculture was harvested at 10⁷ CFU/mL. For coculture (STLP), ST and LP were added at 0.5 × 10⁷ CFU/mL each in a 1:1 ratio. Bacteria were centrifuged (4000 × g, 10 min, 4°C) and resuspended in 5 mL phosphate-buffered saline before adding to the protein solution.

2.2.2 | Pea protein extraction

The extraction method was according to the protocol proposed by Emkani et al. (2021) and Emkani et al. (2023). In summary, seeds from the Assas cultivar were initially

cracked using a Rotor Beater Mill SK300 (RETSCH GmbH, Haan, Germany) to break the grains and separate the hull. Subsequently, compressed air blowing was used to remove the hull. The dehulled cracked grains were then milled in the same mill until they reached a particle size of less than 1 mm, followed by sieving ($< 800 \mu\text{m}$) to obtain pea flour. The pea flour was hydrated in water (10% w/w), and the pH was adjusted by NaOH (0.5 M) to 7.5. The pH was readjusted to 7.5 after allowing the solution to stir overnight. Insoluble material was eliminated through centrifugation ($10,000 \times g$, 30 min, 20°C), and the resulting supernatant (protein solution) was collected. To separate the globulin fraction from the albumin fraction, acidification was carried out until reaching the isoelectric point of globulins at pH 4.8. The reduction of pH was achieved either through the addition of HCl (0.5 M) (control) or through fermentation. The selected LAB were carefully introduced to the protein solution either as a mono- (ST or LP) or coculture (STLP). Although the pH was continuously monitored, the protein solution was incubated at the optimal temperature for the respective bacteria strains: 37°C for LP, 43°C for ST, and 40°C for STLP. The acidification process was concluded once the pH reached 4.8, aligning with the globulins' isoelectric point. The pH reduction occurred more rapidly in the coculture, taking approximately 4–5 hours, compared with the monoculture, which took approximately 9–10 hours (Emkani et al., 2023). The final concentration of the bacteria at the end of the fermentation was around 10^9 CFU/mL. Subsequently, the albumin fraction (A), representing the soluble portion, was separated from the nonsoluble components through centrifugation ($10,000 \times g$, 30 min, 4°C) and carefully collected. The resulting pellet primarily contained globulins along with biomass. To isolate the globulin-rich fraction (G), the pellet was solubilized in water (5% w/v), and the pH was adjusted to 7.5 using 0.5 M NaOH. After overnight stirring and pH readjustment to 7.5, the solution underwent another round of centrifugation ($10,000 \times g$, 20 min, 20°C), followed by the collection of the supernatant. The samples were kept at -20°C prior to analysis.

2.2.3 | Extraction of the volatile compounds

The volatile compounds of albumin and globulin were extracted by the SAFE technique as described by Karolkowski et al. (2023). This technique was opted over others for several reasons, primarily due to its effectiveness in isolating the maximum number of volatile compounds. Additionally, the SAFE method allowed for a single extraction process for nine judges in OAC analysis via GC-O, thereby minimizing result variability. The SAFE method (hydro-distillation) necessitates the dissolution

of hexanal (internal standard) and the matrix in water. Hence, sample preparation was started by mixing 70 mL of ultra-pure water (MilliQ system, Millipore, Bedford, MA, USA) with 200 μL of an internal standard (hexanal D12 in water, 109.8 ng/ μL) (CAS 66-25-1; 96% pure; CDN Isotopes, Cluzeau Info Lab, Sainte Foy La Grande, France) for 30 min on ice, to ensure complete solubilization. Then, 50 g of sample was added and stirred for 10 min. The mixture was then transferred to the dropping funnel of the SAFE apparatus where the entire sample was allowed to flow into the flask over 40 min at 8×10^{-3} mbar, and vacuum distillation (5×10^{-2} mbar) was performed for 45–75 min at 30°C under stirring. The vacuum facilitates the extraction process, allowing to effectively extract the compounds using water as the solvent. By employing this approach, the amount of dichloromethane utilized was significantly reduced. The resulting frozen hydro-distillate reached room temperature. Then, the liquid extraction was performed with dichloromethane (CH_2Cl_2) as the solvent (Carlo Erba, Val de Reuil, France; purity $> 99.9\%$, distilled just before use). Three successive 15-min extraction steps were carried out under stirring using 3×15 mL of CH_2Cl_2 in a water-ice bath, and the recovered organic extracts were pooled and dried over anhydrous Na_2SO_4 (4 g). The extract was then filtered through glass wool before being concentrated to 300 μL (the adjusted volume obtained using CH_2Cl_2) by using two successive Kuderna-Danish apparatuses (Merck, Darmstadt, Germany) of decreasing size equipped with a Snyder column. The extracts were obtained in triplicate for each fraction sample and stored at -20°C before analysis.

2.2.4 | Separation and identification of volatile compounds by GC-MS

A 1- μL volume of each SAFE extract was analyzed by liquid injection into an Agilent 7890A GC (Hewlett-Packard, Palo Alto, CA, USA) equipped with a fused-silica capillary DB-Wax column (30 m \times 0.25 mm id., 0.5- μm film thickness) (Agilent J & W, Santa Clara, CA, USA) with a constant helium flow of 1.2 mL/min. The oven temperature was held at 40°C and then programmed to increase to 240°C at a rate of $4^\circ\text{C}/\text{min}$ and held at this temperature for 10 min. The volatile compounds were identified using a 5975C mass selective detector (Agilent J & W). Mass spectra were taken at 70 eV over a scan range between 29 and 350 amu. Data were acquired using ChemStation software (ver. A.03.00, Agilent J & W). The volatile compounds were identified by comparing their mass spectra with mass spectra libraries (the Wiley 138, NIST, and INRAE databases achieved using standard compounds) and comparing the linear retention index (LRI) calculated using a series of alkanes (C10–C30)

with the LRI from published data (NIST, 2022). Furthermore, in cases where standards from our assortment of aroma compounds were accessible, their mass spectra and LRIs were acquired using a comparable DB-Wax column to validate the identification of volatile compounds. Semi-quantitative data for each compound were obtained by automatic or manual integration of the total ion count peak area (arbitrary units) and expressed as a concentration ($\mu\text{g/g}$ of DM) based on the concentration of a hexanal D12 standard of $109.8 \text{ ng}/\mu\text{L}$.

2.2.5 | Identification of OACs by GC-O

For each fraction, 3 SAFE extracts were mixed to obtain a homogenous sample. Two microliters of the final extracts was injected into an Agilent 6890A GC (Hewlett-Packard) coupled to a flame ionization detector (FID) and an olfactometric detection port (ODP) that constituted the GC-O system. The same column (fused-silica capillary DB-Wax) and chromatographic conditions as GC-MS analysis was used for GC-O. The column was equipped with a Y-splitter to divide the effluent between the FID and ODP (in a 1:1 ratio). Humidified air ($25 \text{ mL}/\text{min}$) was added to the transfer line to prevent nasal mucosa dehydration. LRIs were calculated by weekly injection of a reference solution of n-alkanes (C10 to C30; Sigma-Aldrich, St. Louis, MO, USA) according to Van Den Dool and Kratz (1963).

This study was performed following the relevant institutional and national regulations and legislation (Inserm (French National Institute for Health and Medical Research) Ethic Evaluation Committee, N°21-773, approved the 09/02/2021). Participants were requested to sign an informed consent form and were not informed of the aims of the experiment. They were compensated $\text{€}10$ per hour. Nine assessors who were both sensitive to odor and experienced in the use of GC-O described the odor of the detected area. During a 40-min analysis, the assessors were asked to describe the perceived odor in their own words. Data were acquired using Openlab software (6850/6890 GC system, V2.3, Agilent Technologies) for the chromatography study and an ODP recorder (Gerstel, Mülheim an der Ruhr, Germany) for the descriptive study (a button and a microphone were used to record the description of odor events). The data were processed based on the detection frequency (DF) method following Pollien et al. (1997). OAC areas were characterized in terms of (i) the number of assessors that simultaneously detected an OAC at the ODP (corresponding to the DF); (ii) the odor described by the entire panel in accordance with the literature (INRASnif (internal database), VCF online (www.vcf-online.nl), The Good Scents Company (www.thegoodscentscompany.com), and Fenaroli's Handbook of

Flavor Ingredients (Burdock, 2016); and (iii) the LRI for the column (NIST, 2022). An OA was determined to be odor-active if the DF was higher than 44% ($\geq 4/9$ assessors) (Deuscher et al., 2020; Paravisini et al., 2012). The descriptors were assorted into 9 odor classes of lactic, fruity, floral, chemical, mushroom, grilled, green, potato, and metallic based on literature (Chigwedere et al., 2022; Karolkowski et al., 2023; Roland et al., 2017).

2.2.6 | Statistical analysis

All the statistical treatments were performed using the software package XLSTAT (Addinsoft, Paris, France). The significant differences in the volatile compounds content between the samples obtained without or with fermentation were evaluated by one-way analysis of variance using XLSTAT software. Tukey's post hoc least-significant differences method was used to describe means with 95% confidence intervals.

Principal component analysis (PCA—Pearson correlation) was performed on the volatile compounds quantified by GC-MS as the variable and their chemical classes as supplementary variables. Any volatile compound that corresponded to at least one coelution was excluded from the samples. Moreover, only volatile compounds with significantly different concentrations ($p < 0.05$; Tukey's test) were considered in the construction of the PCA. The PCA was initially performed with albumin and globulin samples together. Another PCA (Pearson correlation) was performed on the 9 odor classes to highlight differences in odor attributes between the samples obtained without or with fermentation. For each odor class, the DF of volatile compounds higher than 44% ($\geq 4/9$ assessors) (a percentage that defined a compound as odor-active) was summed. To clarify different behaviors among samples, PCA considering only albumin or globulin samples was also performed for both GC-MS and GC-O.

3 | RESULTS

3.1 | Identification of volatile compounds by GC-MS

Volatile compounds of albumin (A) and globulin (G) fractions obtained without (Control) or with fermentation by LP, ST, and STLP from pea flour were characterized by GC-MS (Table 1). A total of 96 volatile compounds in pea albumin and globulin fractions were identified by GC-MS. The most representative chemical classes in all the samples included ketones, alcohols, acids, aldehydes, esters, and lactones. Although only a few terpenes,

TABLE 1 Volatile compounds in pea albumin (A) and globulin (G) fractions obtained without (control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP) identified by GC-MS.

Aroma compounds	CAS ^a	LRI _{exp} ^a	LRI _{lit} ^a	Origin	Standard equivalent concentration (µg/g of DM) ^b								Log Pow ^c	Reliability ^d	References
					A-control	G-control	A-LP	G-LP	A-ST	G-ST	A-STLP	G-STLP			
Acids															
Acetic acid	64-19-7	1450	1452	AA ⁷	7.77de ± 1.22	1.74e ± 0.24	23.53c ± 3.54	1.73e ± 0.24	194.44a ± 1.18	2.03e ± 0.52	21.43cd ± 5.10	45.69b ± 0.23	<0.0001	MS, LRI, I	1, 2, 3
Propanoic acid	79-09-4	1540	1556	AA ¹¹	11.21b ± 0.99	3.84d ± 0.34	3.40d ± 0.62	4.92cd ± 0.65	8.55bc ± 0.69	12.35d ± 0.52	8.81bc ± 0.85	34.49a ± 3.82	0.004	MS, LRI, I	1
Butanoic acid	107-92-6	1635	1628		6.63b ± 0.24	10.63b ± 2.45	17.22ab ± 9.47	7.78b ± 1.72	16.71ab ± 2.44	8.42b ± 0.43	7.46b ± 0.24	22.81a ± 2.44	0.135	MS, LRI, I	4
2-Methylbutanoic acid	116-53-0	1670	1674	AA ³	2.62d ± 0.24	ND	67.44a ± 2.44	ND	38.66b ± 1.23	ND	8.75c ± 0.80	ND	0.030	MS, LRI	2, 5, 6
Pentanoic acid	109-52-4	1749	1763		9.87b ± 0.24	0.42d ± 0.024	2.81cd ± 0.24	0.81d ± 0.024	5.09c ± 0.24	1.21d ± 0.024	3.75c ± 0.24	13.84a ± 2.44	0.001	MS, LRI, I	1, 4, 5
Hexanoic acid	142-62-1	1869	1866	FA ⁵	9.61d ± 0.18	7.25de ± 1.15	28.29b ± 2.44	3.43e ± 0.76	38.46a ± 2.44	3.68e ± 0.99	21.03c ± 0.16	8.73d ± 2.44	<0.0001	MS, LRI, I	2, 4, 5, 7
Octanoic acid	124-07-2	2081	2084	FA ³	7.77bc ± 1.34	1.81d ± 0.12	10.39ab ± 2.04	2.99d ± 0.30	13.96a ± 2.92	3.66cd ± 0.67	11.24a ± 1.86	0.65d ± 0.061	<0.0001	MS, LRI, I, O	1, 2, 4, 5, 11
Nonanoic acid	112-05-0	2180	2180	FA ³	6.15c ± 0.24	20.96c ± 1.04	129.94a ± 1.91	10.47c ± 0.32	56.04b ± 2.44	2.85c ± 0.26	61.39b ± 2.44	156.91a ± 2.44	0.007	MS, LRI, O	1, 2, 4, 5
Decanoic acid	334-48-5	2301	2303		2.65cd ± 0.79	1.50d ± 0.20	4.94bc ± 0.31	0.56d ± 0.09	12.69a ± 1.96	2.52cd ± 0.61	4.26bc ± 0.053	5.73b ± 1.25	<0.0001	MS, LRI, I	2, 4
Total:					64.28	48.15	287.96	32.69	384.6	36.72	148.12	288.85			
Alcohols															
2-Methylpropan-1-ol	78-83-1	1091	1092	AA ¹	0.74c ± 0.08	0.80c ± 0.02	3.61a ± 0.51	0.81c ± 0.09	1.29c ± 0.07	Trace	2.30b ± 0.03	1.01c ± 0.15	<0.0001	MS, LRI, I, O	9, 10
1-Methoxypropan-2-ol	107-98-2	1129	1135		1.64c ± 0.10	10.26a ± 1.64	5.84b ± 1.35	2.72c ± 0.64	1.11c ± 0.08	3.14c ± 0.57	2.60c ± 0.60	2.95c ± 0.25	<0.0001	MS, LRI	
Pent-1-en-3-ol	616-25-1	1160	1161	FA ¹	1.79de ± 0.32	12.39a ± 2.52	3.92cd ± 0.33	3.97cd ± 0.66	5.08bc ± 0.64	7.86b ± 3.68	2.54cde ± 0.47	0.93e ± 0.27	<0.0001	MS, LRI, I	4, 5, 7, 8, 9
3-Methylbutan-1-ol	123-51-3	1210	1211	AA ¹	ND	3.17b ± 0.57	17.57a ± 3.45	5.85b ± 0.95	14.14a ± 1.53	5.35b ± 0.50	13.34a ± 2.21	5.97b ± 1.20	<0.0001	MS, LRI, O	5, 9
3-Methylbut-3-en-1-ol	763-32-6	1250	1250		ND	ND	0.75b ± 0.12	0.178b ± 0.01	33.62a ± 1.48	0.45b ± 0.12	0.64b ± 0.12	0.061b ± 0.01	<0.0001	MS, LRI	5
Pentan-1-ol	71-41-0	1253	1252	FA ²	Trace	33.38a ± 5.10	17.81c ± 4.91	19.18c ± 2.67	31.52ab ± 0.94	19.43c ± 2.28	22.59bc ± 1.41	8.87d ± 2.37	<0.0001	MS, LRI, I	1, 4, 5, 7, 8, 9, 11, 13
(Z)-Pent-2-en-1-ol	1576-95-0	1322	1322	FA ¹	2.04d ± 0.24	23.87a ± 3.60	10.17c ± 0.58	16.86b ± 1.68	16.50b ± 0.68	17.86b ± 2.36	14.23bc ± 0.37	3.35d ± 0.24	<0.0001	MS, LRI, I	4, 5, 7
Hexan-1-ol	111-27-3	1356	1356	FA ¹	23.75cd ± 4.31	20.79cd ± 4.62	116.68a ± 15.84	69.64b ± 30.70	10.61cd ± 1.80	8.90cd ± 0.62	25.10c ± 4.71	4.36d ± 0.34	<0.0001	MS, LRI, I	1, 4, 5, 7, 8, 9, 13
(Z)-Hex-3-en-1-ol	928-96-1	1388	1386	FA ¹	2.78d ± 0.32	1.03d ± 0.15	22.80a ± 3.63	7.87c ± 0.88	8.50c ± 0.71	0.90d ± 0.05	14.64b ± 1.54	1.22d ± 0.12	<0.0001	MS, LRI, I, O	4, 5, 7

(Continues)

TABLE 1 (Continued)

Aroma compounds	CAS ^a	LRI _{exp} ^a	LRI _{lit} ^a	Origin	Standard equivalent concentration (µg/g of DM) ^b										Log Pow ^c	P value	Reliability ^d	References
					A-	G-	A-	G-	A-	G-	A-	G-	A-	G-				
Oct-1-en-3-ol	3391-86-4	1452	1456	FA ¹	0.082e ± 0.00	10.47a ± 0.83	2.79c ± 0.24	3.77c ± 0.70	0.61de ± 0.02	4.94b ± 0.03	0.57de ± 0.02	1.16de ± 0.02	<0.0001	MS, LRI, I	1, 4, 5, 7, 8, 9, 11, 13			
2-Ethylhexan-1-ol	104-76-7	1492	1492		9.59e ± 1.82	30.83 ab ± 6.18	10.63de ± 0.86	23.09bc ± 3.04	20.20 cd ± 1.24	35.28a ± 4.88	9.85e ± 0.55	21.77c ± 4.19	<0.0001	MS, LRI	1, 4, 7			
Octan-1-ol	111-87-5	1561	1561	FA ¹	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	3	MS, LRI, I	1, 4, 5, 8, 9			
(E)-Oct-2-en-1-ol	18,409-17-1	1618	1620	FA ¹	ND	ND	ND	3.37a ± 0.74	ND	3.21a ± 0.57	ND	ND	0.793	MS, LRI, I	1, 5, 7, 8			
α-Terpineol	98-55-5	1699	1698		Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2.9	MS, LRI, I, O	6			
Undecan-2-ol	1653-30-1	1721	1717		ND	ND	ND	2.45 ± 0.66	ND	ND	ND	Trace	4.7	MS, LRI, I				
Decan-1-ol	112-30-1	1765	1769		Trace	0.47c ± 0.03	1.64a ± 0.24	0.61c ± 0.12	0.48c ± 0.01	Trace	0.74c ± 0.01	1.16b ± 0.21	0.043	MS, LRI, I	3, 7			
Benzenemethanol	100-51-6	1879	1877	AA ⁷	3.31e ± 0.85	7.23de ± 1.33	24.92a ± 4.60	10.37 cd ± 2.33	19.98ab ± 2.78	6.47de ± 0.35	25.41a ± 1.27	15.70bc ± 0.83	<0.0001	MS, LRI, I, O	1, 4, 5, 13			
2-Phenylethanol	60-12-8	1915	1912	AA ⁷	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	1.3	MS, LRI, I, O	1, 4, 9			
Dodecan-1-ol	112-53-8	1970	1970		3.69b ± 0.72	6.14ab ± 1.48	4.01b ± 0.87	6.14ab ± 1.51	4.42b ± 0.86	5.51ab ± 1.28	3.89b ± 0.75	7.66a ± 0.24	0.292	MS, LRI, I	3, 4, 12			
Phenol	108-95-2	2003	2004	AA ⁶	± 0.08	0.61c ± 0.09	13.42a ± 2.33	0.35c ± 0.02	4.46b ± 1.33	0.35c ± 0.02	0.77c ± 0.02	Trace	0.003	MS, LRI, I	1, 5			
2-Phenoxyethanol	122-99-6	2144	2142		± 0.21	2.75bc ± 0.53	3.16bc ± 0.38	1.09c ± 0.01	5.41b ± 1.12	1.20c ± 0.01	22.33a ± 3.62	3.45bc ± 0.12	<0.0001	MS, LRI, I	5, 10			
Tetradecan-1-ol	112-72-1	2175	2175		± 0.22	3.66a ± 0.24	3.38ab ± 0.27	3.26ab ± 0.63	3.00abc ± 0.33	3.57a ± 0.38	2.50bcd ± 0.40	2.16 cd ± 0.24	0.000	MS, LRI, I				
Hexadecan-1-ol	36,653-82-4	2381	2379		± 6.62	30.80a ± 6.62	6.37b ± 1.75	5.70b ± 0.73	7.11b ± 1.14	9.79b ± 1.03	8.97b ± 1.19	10.55b ± 2.71	<0.0001	MS, LRI, I	10			
Octadecan-1-ol	112-92-5	2588	2585		± 3.26	14.71ab ± 3.26	9.45bc ± 1.27	8.48c ± 0.84	13.83abc ± 2.27	12.15abc ± 1.41	15.36a ± 3.57	10.48abc ± 1.40	0.003	MS, LRI, I				
Total:					101.87	184.72	278.76	195.75	201.87	146.36	188.37	102.81						
Aldehydes																		
Hexanal	66-25-1	1085	1083	FA ¹	± 0.35	2.24cde ± 0.35	13.01a ± 2.96	0.32e ± 0.00	4.40bcd ± 0.79	2.53cde ± 0.64	6.48b ± 0.99	1.13de ± 0.22	4.69bc ± 0.15	0.007	MS, LRI, I, O	1, 2, 3, 4, 5, 7, 8, 11, 13		
Heptanal	111-71-7	1187	1188	FA ¹	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2.2	MS, LRI, I	2, 4, 5, 7, 8, 11		
2-Ethylhexanal	123-05-7	1190	1198		± 2.74	14.29c ± 2.74	146.60a ± 2.13	33.48c ± 1.22	65.70b ± 0.86	66.63b ± 0.47	16.53c ± 0.56	19.77c ± 1.22	0.006	MS, LRI, I	5, 8			
3-Methylbuten-2-al	107-86-8	1201	1206		Trace	Trace	0.92 cd ± 0.12	1.44abc ± 0.05	1.96ab ± 0.55	2.14a ± 0.27	0.65d ± 0.15	1.29bcd ± 0.22	0.000	MS, LRI	10			
(E)-Hexen-2-al	6728-26-3	1221	1219	FA ¹	Trace	0.62b ± 0.03	Trace	1.86a ± 0.10	Trace	1.93a ± 0.17	Trace	Trace	0.000	MS, LRI	1, 4, 5, 7, 8, 9, 14			
Octanal	124-13-0	1292	1291	FA ¹	± 0.13	2.02acd ± 0.13	9.37a ± 1.96	5.10b ± 0.67	1.94 cd ± 0.27	3.52bc ± 0.11	1.21d ± 0.09	1.37d ± 0.34	0.007	MS, LRI, I, O	1, 3, 4, 7, 8, 9, 12, 13			

(Continues)

TABLE 1 (Continued)

Aroma compounds	CAS ^a	LRI _{exp} ^a	LRI _{lit} ^a	Origin	Standard equivalent concentration (µg/g of DM) ^b										Log Pow ^c	Reliability ^d	References
					A-	G-	A-	G-	A-LP	G-LP	A-ST	G-ST	A-STLP	G-STLP			
Nonanal	124-19-6	1396	1396	FA ¹	5.89b ± 0.30	10.61a ± 0.55	8.93a ± 0.66	8.92a ± 0.57	9.46a ± 0.91	9.54a ± 0.23	5.10b ± 0.29	7.89ab ± 0.27	0.531	MS, LRI, I	1, 3, 4, 5, 7, 9, 11, 12, 13		
(E)-Octen-2-al	2548-87-0	1434	1434	FA ¹	0.34d ± 0.02	1.48b ± 0.38	0.27d ± 0.02	1.37b ± 0.01	0.60 cd ± 0.08	2.61a ± 0.26	0.39d ± 0.06	1.06bc ± 0.27	<0.0001	MS, LRI, I, O	1, 2, 3, 5, 7, 8, 9, 11		
Methional	3268-49-3	1456	1454	AA	Trace	Trace	ND	ND	ND	ND	ND	ND	0.4	MS, LRI, I, O	2, 4, 5, 11		
Decanal	112-31-2	1501	1502	FA ³	4.74b ± 0.66	8.56b ± 1.50	9.33ab ± 1.50	6.19b ± 0.53	13.26a ± 3.20	7.85b ± 2.41	5.16b ± 1.29	8.06b ± 1.48	0.049	MS, LRI, I	3, 5, 9, 12		
Benzaldehyde	100-52-7	1526	1520	AA ¹	1.59d ± 0.35	5.11bc ± 0.95	6.48b ± 0.84	3.65c ± 0.70	1.30d ± 0.01	10.99a ± 0.59	1.15d ± 0.06	12.46a ± 0.96	<0.0001	MS, LRI, I	1, 2, 4, 5, 7, 8, 9		
(2E,4E)-nona-2,4-dienal	6750-03-4	1703	1710	FA ¹	ND	Trace	ND	ND	ND	Trace	ND	ND	2.9	MS, LRI, O	1, 3, 4, 7, 8, 11, 12		
Dodecanal	112-54-9	1712	1710	1.44bc ± 0.30	2.38b ± 0.50	1.57bc ± 0.18	0.67c ± 0.13	1.97b ± 0.24	2.47ab ± 0.15	1.54bc ± 0.24	3.61a ± 0.92	0.082	MS, LRI, I	2, 9			
(2E,4Z)-deca-2,4-dienal	25,152-83-4	1768	1767	FA ¹	ND	Trace	ND	Trace	ND	Trace	ND	ND	3.1	MS, LRI	5, 10, 22		
(2E,4E)-deca-2,4-dienal	25,152-84-5	1814	1824	FA ¹	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	3.1	MS, LRI, O	1, 2, 3, 4, 5, 7, 11		
Tetradecanal	10,486-19-8	1924	1924	1.49b ± 0.22	2.69a ± 0.77	Trace	Trace	Trace	Trace	Trace	Trace	Trace	0.050	MS, LRI, I	5, 7		
4,5-Epoxy-(E)-dec-2-enal	134,454-31-2	2005	2006	FA ⁴	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	1.7	MS, LRI, O	10, 16,		
Pentadecanal	2765_11_9	2030	2042	6.99b ± 0.43	5.45b ± 0.16	4.95b ± 3.05	ND	15.52a ± 0.01	ND	ND	ND	ND	<0.0001	MS, LRI	7		
Total:				41.03	205.88	68.29	99.3	86.65	114.16	32.86	60.2						
Esters																	
Ethyl butanoate	105-54-4	1038	1035	0.21b ± 0.01	0.12b ± 0.01	0.78a ± 0.01	0.17b ± 0.01	0.72a ± 0.01	0.15b ± 0.01	0.60a ± 0.00	0.23b ± 0.00	0.000	1.4	MS, LRI, I	18		
Ethyl 2-hydroxypropanoate	97-64-3	1345	1349	ND	ND	55.12b ± 7.75	24.23c ± 3.24	12.21c ± 1.85	6.83c ± 1.51	86.53a ± 2.14	26.43c ± 3.24	<0.0001	0.1	MS, LRI, I	5		
6-Methylheptyl prop-2-enoate	54,774-91-3	1487	1480	0.63d ± 0.12	9.69a ± 2.45	Trace	5.13bc ± 0.99	Trace	3.35 cd ± 0.61	Trace	8.59ab ± 1.15	0.007	3.9	MS, LRI	19		
Ethyl 2-hydroxy-4-methylpentanoate	10,348-47-7	1545	1545	ND	ND	0.35b ± 0.01	ND	ND	ND	1.75a ± 0.12	ND	<0.0001	1.3	MS, LRI			
Dimethyl glutarate	1119-40-0	1700	1699	ND	ND	ND	ND	56.84a ± 1.22	ND	38.93b ± 0.24	ND	<0.0001	0.4	MS, LRI	20		
Isopropyl myristate	110-27-0	2036	2041	6.40 cd ± 0.89	12.11bc ± 2.88	19.66ab ± 3.28	2.66d ± 0.60	19.71ab ± 5.69	11.34c ± 2.39	20.36a ± 2.92	11.81bc ± 2.33	<0.0001	7.7	MS, LRI, I	10, 21		
Isopropyl palmitate	142-91-6	2239	2232	1.75 cd ± 0.20	4.13a ± 0.32	1.41de ± 0.26	1.58 cd ± 0.06	2.12bc ± 0.00	0.83e ± 0.00	2.57b ± 0.32	4.33a ± 0.47	0.165	8.2	MS, LRI, I			
Methyl dihydrojasmonate	24,851-98-7	2290	2276	2.13b ± 0.16	2.10b ± 0.58	0.86b ± 0.11	1.84b ± 0.19	1.17b ± 0.26	1.91b ± 0.27	1.08b ± 0.01	4.88a ± 1.22	<0.0001	2.6	MS, LRI, I, O			

(Continues)

TABLE 1 (Continued)

Aroma compounds	CAS ^a	LRI _{exp} ^a	LRI _{lit} ^a	Origin	Standard equivalent concentration (µg/g of DM) ^b										Log Pow ^c	Reliability ^d	References
					A-	G-	A-LP	G-LP	A-ST	G-ST	A-STLP	G-STLP	P value				
Total:		11.12	28.15	78.18	35.61	92.77	24.41	151.82	56.27								
Ketones																	
3,3-Dimethylbutan-2-one	75-97-8	964	978	978	6.62b ± 1.05	5.52bc ± 1.18	9.06a ± 1.53	4.26 cd ± 0.48	4.95bc ± 0.36	2.35d ± 0.10	4.13 cd ± 0.74	<0.0001	1.2	MS, LRI			
Butane-2,3-dione	431-03-8	980	977	CAR ⁶	1.37d ± 0.01	2.24d ± 0.04	0.40d ± 0.07	280.62c ± 1.94	12.96d ± 1.07	824.52a ± 6.77	389.30b ± 7.56	<0.0001	-1.3	MS, LRI, I, O	7, 9		
Pentan-2-one	107-87-9	985	986		3.51abc ± 0.14	2.85ab ± 0.19	3.69abc ± 0.05	2.81bc ± 0.12	4.59a ± 0.54	2.50c ± 0.12	4.06a ± 0.25	0.597	0.9	MS, LRI, I	9, 10, 14		
4-Methylpentan-2-one	108-10-1	1009	1008		1.01b ± 0.09	0.48c ± 0.01	1.31b ± 0.16	0.64c ± 0.13	1.12b ± 0.01	0.34c ± 0.01	0.40c ± 0.01	0.014	1.3	MS, LRI, I			
Pentane-2,3-dione	600-14-6	1057	1058	FA ³	ND	2.66a ± 0.28	Trace	Trace	0.36b ± 0.06	Trace	0.19b ± 0.01	<0.0001	-0.8	MS, LRI, I, O	4, 5, 7, 9		
Heptan-3-one	106-35-4	1156	1163		5.26 cd ± 0.80	19.46a ± 4.84	3.78 cd ± 1.42	7.25bcd ± 1.12	10.45bc ± 1.34	3.99d ± 0.15	11.93b ± 0.10	0.039	1.7	MS, LRI			
Heptan-2-one	110-43-0	1185	1185		2.38bc ± 0.58	1.78c ± 0.28	5.53ab ± 2.14	6.77a ± 0.48	6.43a ± 0.67	7.47a ± 0.90	7.47a ± 1.97	<0.0001	1.9	MS, LRI, I	1, 4, 5, 7, 8, 9, 11, 13		
6-Methylheptan-2-one	928-68-7	1241	1237	CAR ²	2.97b ± 0.27	11.51a ± 0.96	4.06b ± 0.42	5.88ab ± 0.46	7.07ab ± 1.71	3.92b ± 1.00	3.82b ± 1.68	0.201	2.3	MS, LRI, I, O	10		
5-Methylheptan-2-one	18,217-12-4	1259	1256		1.34c ± 0.01	6.46a ± 1.11	2.63bc ± 0.52	2.31bc ± 0.43	2.04bc ± 0.44	1.56bc ± 0.25	2.05bc ± 0.31	0.070	2.3	MS, LRI			
3-Hydroxybutan-2-one	513-86-0	1287	1287		2.89c ± 0.26	10.23c ± 0.24	129.5bc ± 22.03	28.60c ± 2.26	436.75b ± 184.05	1456.05a ± 263.52	1477.91a ± 183.26	<0.0001	-0.3	MS, LRI, I, O	5, 11		
Octan-2-one	111-13-7	1288	1283		0.62b ± 0.06	1.83a ± 0.44	ND	ND	ND	ND	ND	0.006	2.3	MS, LRI, I	1, 4, 5, 7, 8, 9		
1-Hydroxypropan-2-one	116-09-6	1301	1290		2.00b ± 0.47	1.24b ± 0.10	5.96a ± 1.20	1.46b ± 0.30	1.90b ± 0.25	5.58a ± 0.57	2.02b ± 0.35	<0.0001	-0.7	MS, LRI, I			
Oct-1-en-3-one	4312-99-6	1304	1306	FA ²	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2.1	MS, LRI, I, O	8, 17		
3-Methylcyclohexanone	591-24-2	1334	1333		0.59c ± 0.12	1.28a ± 0.16	Trace	0.65c ± 0.15	0.99ab ± 0.15	Trace	0.76bc ± 0.06	0.002	1.7	MS, LRI, I			
Nonan-2-one	821-55-6	1392	1391		1.55d ± 0.24	2.87bcd ± 0.51	1.98 cd ± 0.39	3.54bc ± 0.58	3.92b ± 0.68	3.14bcd ± 0.50	6.34a ± 1.15	0.093	3.1	MS, LRI, I	1, 4, 8, 9, 13		
Oct-3-en-2-one	1669-44-9	1411	1414		Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2.5	MS, LRI, O	3, 4, 5, 8		
Decan-2-one	693-54-9	1496	1493		1.28bc ± 0.06	1.00c ± 0.13	3.37a ± 0.86	0.84c ± 0.20	0.76c ± 0.14	3.26a ± 0.49	0.58c ± 0.11	<0.0001	3.7	MS, LRI, I	1, 5, 7, 8, 13		
(3E,5E)-octa-3,5-dien-2-one	30,086-02-3	1574	1570	FA ¹	Trace	3.14a ± 0.38	Trace	0.77c ± 0.05	2.47b ± 0.27	Trace	Trace	<0.0001	1.7	MS, LRI, I	1, 2, 3, 5, 7, 8, 9, 13		
Undecan-2-one	112-12-9	1601	1599		1.01c ± 0.19	1.90c ± 0.35	5.06a ± 0.19	3.45b ± 0.47	4.69ab ± 1.17	1.12c ± 0.21	4.67ab ± 0.70	0.018	4	MS, LRI, I	1, 2, 3, 5, 7, 8		
Acetophenone	98-86-2	1655	1652	AA ¹	1.12c ± 0.28	3.99a ± 0.80	2.30bc ± 0.53	1.23c ± 0.06	3.26ab ± 0.68	2.21bc ± 0.19	4.53a ± 0.46	0.014	1.5	MS, LRI, I	1, 9		

(Continues)

TABLE 1 (Continued)

Aroma compounds	CAS ^a	LRI _{exp} ^a	LRI _{lit} ^a	Origin	Standard equivalent concentration (µg/g of DM) ^b										Log Pow ^c	P value	Reliability ^d	References
					A-control	G-control	A-LP	G-LP	A-ST	G-ST	A-STLP	G-STLP	A-STLP	G-STLP				
Tridecan-2-one	593-08-8	1812	1814		1.38 cd ± 0.16	1.24 d ± 0.00	1.75 cd ± 0.35	5.27 a ± 0.78	1.37 cd ± 0.19	2.58 bc ± 0.45	1.00 d ± 0.13	3.58 b ± 0.44	<0.0001	5	MS, LRI, I	4, 7		
Geranylacetone	3796-70-1	1857	1859	CAR ³	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	3.6		MS, LRI, I, O	4, 7, 12		
Pentadecan-2-one	2345-28-0	2021	2021		0.93 b ± 0.01	0.64 b ± 0.16	0.68 b ± 0.14	2.63 a ± 0.63	0.47 b ± 0.00	1.44 b ± 0.006	0.91 b ± 0.08	3.68 a ± 0.063	0.005	6	MS, LRI, I			
2-Aminocetophenone	551-93-9	2220	2218		Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	1.6		MS, LRI, O			
Total:					35.99	83.23	179.5	88.35	2134.66	508.73	2318.85	6596.47						
Lactones																		
γ-Pentalactone	108-29-2	1612	1600		0.90 b ± 0.02	Trace	1.01 b ± 0.06	Trace	0.96 b ± 0.21	ND	2.05 a ± 0.49	Trace	0.009	-0.2	MS, LRI, I	5, 10		
γ-Butyrolactone	96-48-0	1630	1626		1.79 cd ± 0.29	1.51 d ± 0.39	6.16 a ± 0.88	2.47 cd ± 0.12	2.58 cd ± 0.10	3.17 c ± 0.10	3.47 bc ± 0.43	4.88 ab ± 1.13	<0.0001	-0.7	MS, LRI, I	5, 10		
γ-Hexalactone	695-06-7	1705	1708		Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	0.4		MS, LRI, I	4, 5, 10		
γ-Crotonolactone	497-23-4	1755	1745		0.56 c ± 0.09	1.16 b ± 0.09	2.22 a ± 0.16	1.44 b ± 0.33	0.44 c ± 0.06	2.22 a ± 0.31	1.01 bc ± 0.16	1.55 b ± 0.32	<0.0001	-0.6	MS, LRI	10		
γ-Octalactone	104-50-7	1922	1923	FA ³	0.28 b ± 0.02	0.39 b ± 0.02	0.69 b ± 0.02	0.33 b ± 0.02	0.52 b ± 0.02	0.32 b ± 0.02	0.41 b ± 0.02	2.62 a ± 0.50	<0.0001	1.43	MS, LRI, I, O	2, 5, 8		
γ-Nonanolactone	104-61-0	2033	2037	FA ³	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2		MS, LRI, I, O	3, 4, 5, 7, 8		
γ-Decalactone	706-14-9	2150	2152		0.43 b ± 0.00	0.24 d ± 0.02	0.45 b ± 0.01	0.29 d ± 0.04	0.43 b ± 0.01	0.37 c ± 0.03	0.45 b ± 0.00	1.00 a ± 0.28	0.05	2.7	MS, LRI, I, O	10, 16		
δ-Decalactone	705-86-2	2202	2201	FA ¹⁰	ND	ND	5.03 a ± 1.23	1.04 b ± 0.11	3.87 a ± 0.69	1.20 b ± 0.18	4.33 a ± 0.65	3.29 a ± 0.28	0.06	2.4	MS, LRI, I, O	15		
δ-Jasmine lactone	25-524-95-2	2266	2272	FA ⁹	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	Coelution	2.1		MS, LRI, O			
Total:					3.96	3.3	15.56	5.57	8.8	7.28	11.72	13.34						
Terpenes																		
Verbenone	80-57-9	1718	1710		Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	2.1		MS, LRI, I, O	10		
(E)-Geraniol	106-24-1	1850	1853		ND	ND	5.15 ab ± 0.69	6.85 a ± 1.28	3.90 bc ± 0.84	2.58 c ± 0.19	2.89 c ± 0.76	ND	0.045	3.2	MS, LRI, I, O	3, 7, 10		

(Continues)

TABLE 1 (Continued)

Aroma compounds	CAS ^a	LRI ^a _{exp}	LRI ^a _{lit}	Origin	Standard equivalent concentration (µg/g of DM) ^b					P value	Log Pow ^c	Reliability ^d	References		
					A-control	G-control	A-LP	G-LP	A-ST					G-ST	A-STLP
Thiazole															
Benzothiazole	95-16-9	1963	1968	0.54b ± 0.02	0.99b ± 0.05	0.57b ± 0.13	0.68b ± 0.08	1.28b ± 0.23	1.01b ± 0.25	4.81a ± 0.11	1.62b ± 0.24	<0.0001	2	MS, LRI, I	4, 5
Pyrazines															
2,5-Dimethylpyrazine	123-32-0	1327	1332	AA ⁸	ND	ND	Trace	Trace	Trace	Trace	Trace	Trace	-0.2	MS, LRI, I, O	7, 8

Abbreviations: FA: fatty acids, AA: amino acids, CAR: carotenoids, ND: not detected by GC-MS.

^aCAS and DB-Wax linear retention index (LRI) experimental and from the literature (NIST, 2023).

^bConcentration of aroma compound (presented as µg of internal standard/g of DM) are shown as mean ± standard deviation, *n* (*n* = 3) (different letters represent a significant difference between samples considering the same analysis (*p* < 0.05, Tukey's test)).

^cLog Pow, octanol/water partition coefficient expressed in logarithmic form and extracted from literature (Kim et al., 2023).

^dReliability of identification: MS = mass spectrometry, LRI = linear retention index, O = odor identical to published data and/or data found in databases, and I = confirmation by injection of standards on equivalent DB-Wax.

Origin: ¹(Murray et al., 1976), ²(Jakobsen et al., 1998), ³(Karolkowski et al., 2021), ⁴(Bader et al., 2009), ⁵(Baill et al., 2009), ⁶(Chen et al., 2017), ⁷(Akkad et al., 2019), ⁸(Zhang et al., 2020), ⁹(Haffner et al., 1996), ¹⁰(Albrecht et al., 1992), ¹¹(Nakae & Elliott, 1965).

References: 1 (Xiang et al., 2022), 2 (Utz et al., 2022), 3 (Ebert et al., 2022), 4 (Benavides-Paz et al., 2022), 5 (Murat et al., 2013), 6 (Wang et al., 2020), 7 (Schmidler et al., 2012), 8 (Fischer et al., 2022a), 9 (El Youssef et al., 2020), 10 (Karolkowski et al., 2023), 11 (Trikusuma et al., 2020), 12 (Pei et al., 2022), 13 (Shi et al., 2021), 14 (Ben-Harb et al., 2022), 15 (Masia et al., 2021), 16 (Bader et al., 2009), 17 (Jakobsen et al., 1998), 18 (Akkad et al., 2022), 19 (Rajhi et al., 2022), 20 (Xu et al., 2020), 21 (Zhang et al., 2021), and 22 (Kaneko et al., 2011).

pyrazines, and thiazoles were detected, 68 compounds have already been reported for pea protein or pea flour, 10 have been reported in other pulses, and to the best of the authors knowledge, 16 have not been detected in pea or in the other pulses (Table 1). The majority of volatile compounds were the products of fatty acids and amino acid degradation. Although a few of them were originated from carotenoids belonging to the ketones (Chen et al., 2017; Karolkowski et al., 2021; Murray et al., 1976; Nakae & Elliott, 1965). Some of these volatiles were present in all the samples with no significant differences in their concentration, that is, butanoic acid, dodeca-1-ol, nonanal, dodecanal, isopropyl palmitate, pentan-2-one, 4-methylpentan-2-one, 6-methylheptan-2-one, 5-methylheptan-2-one, nonan-2-one, undecan-2-one, and γ -decalactone. Some of the volatile compounds were detected only in fermented samples, that is, 3-methylbut-3-en-1-ol, (*E*)-oct-2-en-1-ol, undecan-2-ol, ethyl 2-hydroxypropanoate, ethyl 2-hydroxy-4-methylpentanoate, dimethyl glutarate, δ -decalactone, geraniol, 2,5-dimethylpyrazine. However, some others were detected only in controls, that is, methional and octan-2-one.

Comparing the acid volatile compounds in albumin and globulin fractions of control samples, A-Control (64.28 µg/g of DM) had a higher content of total acids compared with G-Control (48.15 µg/g of DM), although a significant difference was observed mainly in pentanoic acid and octanoic acid. Fermented samples (both albumin and globulin samples) had a higher content of acids compared with the controls. Among fermented samples, albumin fractions (~148–384 µg/g of DM) had a higher content of total acids compared with globulin fractions (~32–288 µg/g of DM). A-ST had the highest value in acetic acid, hexanoic acid, octanoic acid, and decanoic acid. In globulin-fermented samples, G-STLP had the highest content of propanoic acid, pentanoic acid, and nonanoic acid. 2-Methylbutanoic acid was not found in G-Control and fermented globulin samples (Table 1).

G-Control (184.72 µg/g of DM) had a higher content of total alcohols compared with A-Control (101.87 µg/g of DM). 1-Methoxypropan-2-ol, pent-1-en-3-ol, pentan-1-ol, (*Z*)-pent-2-en-1-ol, oct-1-en-3-ol, 2-ethyl-1-hexanol, and decan-1-ol had the highest value in G-Control compared with A-Control and fermented samples. 3-Methylbut-3-en-1-ol was not detected in G-Control and A-Control, and it had the highest value in A-ST. (*E*)-oct-2-en-1-ol was present only in G-ST and G-LP. Undecan-2-ol was detected only in G-ST and G-STLP. 3-Methylbutan-1-ol was not present in A-Control, while it had a higher value in all the albumin-fermented samples. 2-Methylpropan-1-ol, hexan-1-ol, (*Z*)-hex-3-en-1-ol, and phenol had the highest value in

A-LP. Benzenemethanol had the highest value in A-STLP and A-LP.

Esters were detected mainly in albumin-fermented samples in particular A-STLP. Ethyl 2-hydroxypropanoate was not found in G-Control and A-Control, and it had the highest value in A-STLP. Isopropyl myristate had the highest content in A-LP, A-ST, and A-STLP. Ethyl butanoate had the highest value in A-ST. Dimethyl glutarate was found only in A-ST and A-STLP. Ethyl 2-hydroxy-4-methylpentanoate was found only in A-STLP and A-LP.

There was no significant difference in the concentration of lactones between albumin and globulin controls, except for γ -pentalactone, which was higher in A-Control. Same as esters, the total content of lactones was higher in fermented samples (~ 5 – 15 $\mu\text{g/g}$ of DM) compared with controls (~ 3 $\mu\text{g/g}$ of DM), especially the ones fermented with STLP and A-LP. γ -Decalactone was detected only in fermented samples. γ -Pentalactone was detected mainly in albumin fraction, and it had the highest value in A-STLP. γ -Octalactone had the highest value in A-STLP, and γ -butyrolactone had the highest value in A-STLP, G-STLP, and A-ST.

Aldehydes seemed to be more abundant in globulin fractions (~ 99 – 205 $\mu\text{g/g}$ of DM). G-Control had the highest value in hexanal, octanal, 2-ethylhexanal, and tetradecanal, compared with A-Control and fermented samples. G-ST seemed to have the highest concentration in (*E*)-hexen-2-al, (*E*)-octen-2-al, and benzaldehyde. (*2E,4E*)-nona-2,4-dienal and (*2E,4Z*)-deca-2,4-dienal were not detected in albumin fractions, and it had only a trace amount in G-Control, G-LP, and G-ST. 3-Methyl-buten-2-al was detected mainly in fermented samples, especially the ones fermented with ST.

G-Control represented a higher content in the majority of ketones, i.e., pentane-2,3-dione, heptan-3-one, octan-2-one, (*3E,5E*)-octa-3,5-dien-2-one, and acetophenone compared with A-Control and albumin and globulin fermented samples. Pentane-2,3-dione was detected mainly in globulin fractions. On the other hand, there was an increase in the content of some other ketones in fermented samples: butane-2,3-dione had the highest concentration in A-STLP, and it was followed by G-STLP. 1-Hydroxypropan-2-one and decan-2-one had the highest concentration in fermented albumin fractions. 3-Hydroxybutan-2-one had the highest content in A-ST, A-STLP, and G-STLP.

There were only two terpenes detected, i.e., verbenone and (*E*)-geraniol. (*E*)-geraniol was not detected in controls and G-STLP samples while it showed the highest concentration in G-LP. Pyrazines generally have lower concentrations in pea protein, and they can be barely detected. Here, 2,5-dimethylpyrazine was not detected in controls and it had a trace quantity in the other samples.

PCA (Figure 1) was performed in order to clarify the

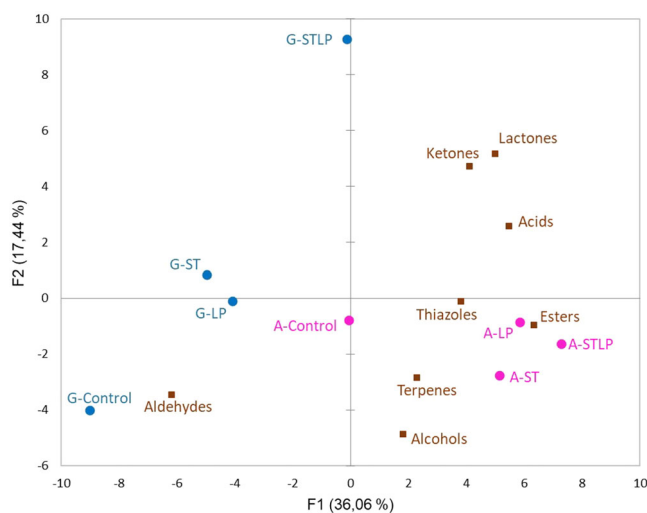


FIGURE 1 Principal component analysis (PCA—Pearson correlation) performed on the volatile compounds ($p < 0.05$) of pea protein albumin (A) and globulin (G) fractions obtained without (control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP) (●). The 8 chemical classes (■) are also shown as the supplementary variable. The volatile compounds with significant differences in equivalent concentration were considered for this analysis ($p \geq 0.05$; Tukey's test).

complex data analysis of volatile compounds among all the samples. The volatile compounds were considered as variable, and the chemical class as a quantitative supplementary variable. The volatile compounds that did not have significant differences ($p \geq 0.05$; Tukey's test) between samples were not considered in PCA. Also, some of the volatile compounds were coeluted and the quantity could not be evaluated. Neither of these compounds were considered in PCA. The first component represented 36.06% of the total variance, and the second component represented 17.44%. The first component separated albumin from globulin fractions and fermented samples from controls. A-ST, A-LP, and A-STLP were characterized mainly by a high content of esters, thiazoles, ketones, lactones, acids, alcohols, and terpenes and a low quantity of aldehydes. Globulin fractions (except G-STLP) were characterized by high quantities of aldehydes, especially G-Control. The second component separated G-STLP from other globulin fractions. G-ST and G-LP showed similar aroma profiles and differentiated from G-STLP. This last sample had a higher content of lactones, ketones, and acids compared with the two other fermented globulin fractions.

To have a better idea of the differences between the samples regarding the strains (mono- and coculture), a second PCA was performed for albumin and globulin fractions separately (Figure 2). For albumin fractions (Figure 2a), the first and second components represented 48.14% and

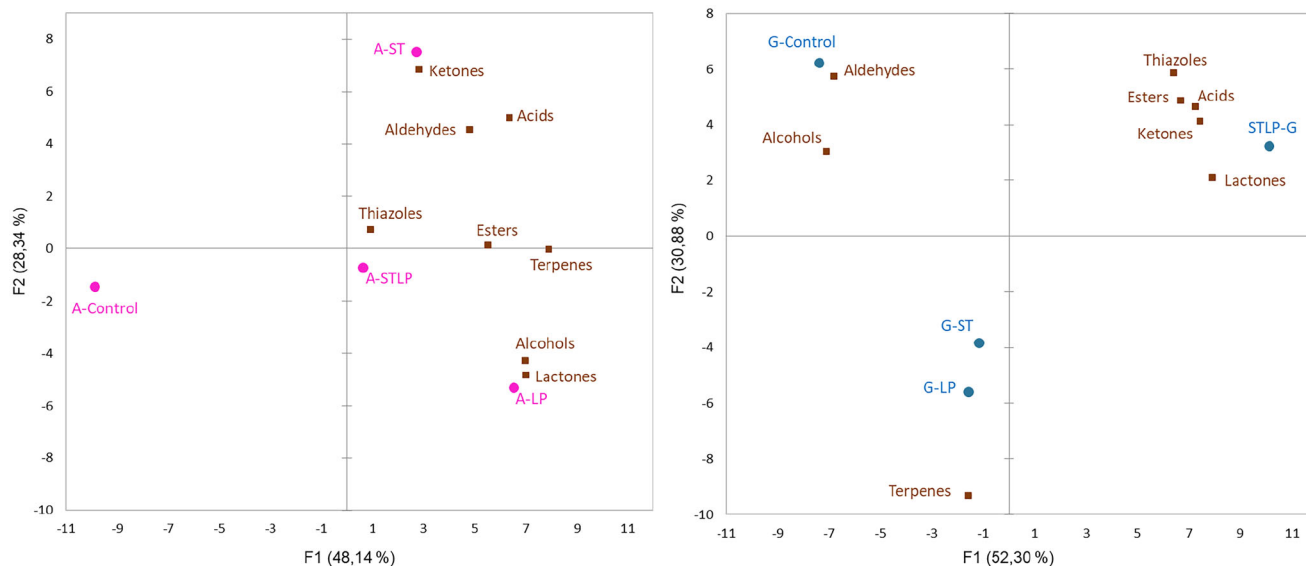


FIGURE 2 Principal component analysis (PCA—Pearson correlation) performed on the volatile compounds ($p < 0.05$) of pea protein albumin (A) (a) and globulin (G) (b) fractions (●) obtained without (control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP). The 8 chemical classes (■) are also shown as the supplementary variable. The volatile compounds with significant differences in equivalent concentration were considered for this analysis ($p \geq 0.05$; Tukey's test).

28.34% of the variance, respectively. The first component separated the fermented albumin samples from the control. Clearly, fermented samples contained a higher quantity of volatile compounds compared with the control. The second component separated albumin-fermented samples depending on the fermenting strain. A-LP contained higher content of lactones and alcohols compared with the two other fermented samples. However, A-ST represented a higher quantity of acids, aldehydes, and ketones. In globulin samples (Figure 2b), the first and second components represented 52.30% and 30.88% of the variance, respectively. The first component separated G-STLP from G-Control. G-STLP had a higher quantity of lactones, ketones, acids, thiazoles, and esters compared with the other samples. On the contrary, G-Control contained a higher quantity of aldehydes and alcohols. The second component separated G-ST and G-LP from G-STLP and G-Control. G-ST and G-LP seemed to have a closer aroma composition to the control sample, although they had a higher content of terpenes compared with the other two samples (representing only 2 volatile compounds identified as already mentioned).

3.2 | Odor-active compounds

Thirty-six OACs were detected by GC-O within the control and fermented samples (Table 2), among which 7 (i.e., ethyl-3-methylbutanoate or ethyl-2-methylbutanoate, hex-1-en-3-one, (*Z*)-non-2-enal,

2-isobutyl-3-methoxy-pyrazine, (*E*)-non-2-enal, 2-acetylpyrazine, and 2,6-dimethoxyphenol) were not detected by GC-MS. It was supposed that the LRI at 1056 with the odor attribute of fruity could correspond to any of ethyl-3-methylbutanoate or ethyl-2-methylbutanoate as LRIs were in accordance with those in the database.

The 36 OACs were classified into 9 odor classes, that is, lactic, fruity, floral, metallic, chemical, green, grilled, potato, and mushroom. Although green, potato, mushroom, and metallic could be all considered as responsible for the beany note in pea protein (Chigwedere et al., 2022), here they were separated to better distinguish between the samples. Some OACs were detected in all the samples, including, hex-1-en-3-one (chemical), oct-1-en-3-one (mushroom), oct-3-en-2-one (mushroom), (*E*)-oct-2-enal (green), and 4,5-epoxy-(*E*)-dec-2-enal (metallic).

Comparing the albumin and globulin fractions obtained as controls, ethyl-3-methylbutanoate or ethyl-2-methylbutanoate (fruity), 2-acetylpyrazine (grilled), geranylacetone (chemical), benzenemethanol (fruity), γ -nonanolactone (fruity), and octanoic acid (chemical) were detected in A-Control. (*Z*)-hex-3-en-1-ol (metallic), 2-isobutyl-3-methoxy-pyrazine (green), and (2*E*,4*E*)-deca-2,4-dienal (grilled) were detected in G-Control. 6-Methylheptan-2-one (chemical) and (2*E*,4*E*)-nona-2,4-dienal (grilled) were perceived only in G-Control, and not in other samples.

Comparing fermented and control samples, some OACs were detected only in fermented samples, that is, butane-2,3-dione (lactic), 2-methylpropan-1-ol (fruity),

TABLE 2 OACs found in pea albumin (A) and globulin (G) fractions obtained without (control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP). The compounds shown in bold here had a detection frequency (DF) of ≥ 4 assessors. The unbold values had a detection frequency (DF) of <4 .

Aroma compounds	LRI(FID) ^a	LRI(MS)	Odor attributes	Odor classes	DF (%)							
					A-control	G-control	A-ST	G-ST	A-LP	G-LP	A-STLP	G-STLP
Butane-2,3-dione	994	980	Butter	Lactic	11	ND	22	11	22	11	78	22
Ethyl-3-methylbutanoate or ethyl-2-methylbutanoate	1056	X	Fruity	Fruity	34	ND	11	ND	ND	ND	45	11
2-Methylpropan-1-ol	1091	1092	Solvent, fruity, acid	Fruity	11	ND	11	ND	ND	ND	11	56
Hex-1-en-3-one	1104	X	Plastic, dust, warm cloth, leather	Chemical	67	56	78	67	67	56	67	67
3-Methylbutan-1-ol	1202	1209	Herbaceous, metallic, plastic, grilled, greenwood	Metallic	ND	11	ND	11	ND	11	56	34
6-Methylheptan-2-one	1241	1241	Rancid, phenol, dust, sulfur	Chemical	22	45	22	34	11	22	22	11
3-Hydroxybutan-2-one	1287	1287	Butter, milk, cream	Lactic	ND	ND	67	34	89	78	78	78
Oct-1-en-3-one	1302	1304	Mushroom	Mushroom	89	89	89	100	89	89	89	89
2,5-Dimethylpyrazine	1340	1327	Roasted peanuts, cereals, rusk, toasted bread	Grilled	ND	11	ND	22	ND	67	ND	ND
(Z)-Hex-3-en-1-ol	1384	1388	Vegetal, Garlic, metallic, burnt hair, unpleasent	Metallic	22	67	56	ND	34	56	22	ND
Oct-3-en-2-one	1405	1411	Mushroom	Mushroom	89	56	89	89	78	67	78	67
(E)-oct-2-enal	1435	1434	Vegetal, nutty, poppy, dust, green	Green	78	89	67	67	78	67	67	45
Methional	1454	1456	Potato	Potato	89	89	78	89	ND	ND	ND	ND
(Z)-Non-2-enal	1509	X	Green, leaves, herb, wood bark, dust	Green	11	ND	11	11	56	22	34	22
2-Isobutyl-3-methoxy-pyrazine	1528	X	Vegetable, bell pepper, green, carrot	Green	11	67	ND	ND	ND	34	ND	45
(E)-Non-2-enal	1537	X	Vegetal, grass, vegetable, faba bean, soup	Green	22	34	34	56	45	34	34	34
2-Acetylpyrazine	1652	X	Cereal, bread, grilled, roasted	Grilled	45	34	34	34	56	45	45	56
(2E,4E)-nona-2,4-dienal	1702	Trace	Meat broth, cereals, grilled chicken, bread	Grilled	11	56	ND	11	11	34	ND	ND
α -Terpineol	1719	1699	Anise, floral, menthol	Floral	22	34	56	34	22	22	11	22
Verbenone	1726	1717	Anise, flower, caramel, violet, fennel	Floral	56	45	34	34	34	56	78	45

(Continues)

TABLE 2 (Continued)

Aroma compounds	LRI(FID) ^a	LRI(MS)	Odor attributes	Odor classes	DF (%)		A-LP	G-LP	A-ST	G-ST	A-STLP	G-STLP
					A-control	G-control						
(2E,4E)-deca-2,4-dienal	1810	1814	Grilled meat, cereals, toasted bread, spice	Grilled	34	56	11	78	45	89	ND	ND
Geraniol	1848	1850	Citrus, orange blossom, verbena, fruity	Fruity	ND	ND	45	34	ND	22	ND	ND
Geranylacetone	1858	1857	Phenolic, pharmaceutical, smoky, burned	Chemical	45	34	67	34	56	ND	56	ND
Benzenemethanol	1870	1879	Sweet, vanilla, coconut, peach, candy-floss	Fruity	56	11	45	11	56	34	45	56
2-Phenylethanol	1913	1915	Floral, rose, grape juice	Floral	22	22	89	78	56	67	78	56
γ -Octalactone	1917	1921	Sugared almonds, rose, candy-floss, floral	Fruity	11	11	ND	ND	11	ND	22	67
4,5-Epoxy-(E)-dec-2-enal	2004	Trace	Meat broth, metallic, beany, Green	Metallic	56	78	45	100	78	78	67	67
γ -Nonanolactone	2026	2033	Fruity, strawberry, raspberry, candy-floss	Fruity	45	22	45	67	56	45	56	67
Octanoic acid	2085	2081	Animal, fatty, candle	Chemical	45	34	67	34	67	34	34	56
γ -Decalactone	2150	2151	Applesauce, fruity, apricot, peach	Fruity	11	22	11	22	ND	11	45	56
Nonanoic acid	2186	2191	Leather, animal, rubber	Chemical	67	67	34	78	45	45	45	34
δ -Decalactone	2202	2203	candy-floss, fruity, apricot, peach	Fruity	11	ND	45	45	67	11	67	56
2-Aminoacetophenone	2221	2218	Floral, honey, candy, orange blossom	Floral	34	11	78	56	67	78	78	56
δ -Jasmine lactone	2268	2266	Sugar, fruity, peach, oil	Fruity	34	11	45	22	78	34	34	45
2,6-Dimethoxyphenol	2274	X	Grilled, chemical, leather, dust, beany, cauliflower	Chemical	56	67	56	56	11	22	ND	11
Methyl dihydrojasmonate	2306	2290	Fruity, mint, anise, fennel	Floral	ND	ND	67	11	56	67	67	56

Abbreviations: ND: not detected by GCO, X: not detected by GC-MS.

^aLRI(FID): linear retention index obtained by flame ionization detector in GCO and LRI(MS) obtained by GC-MS.

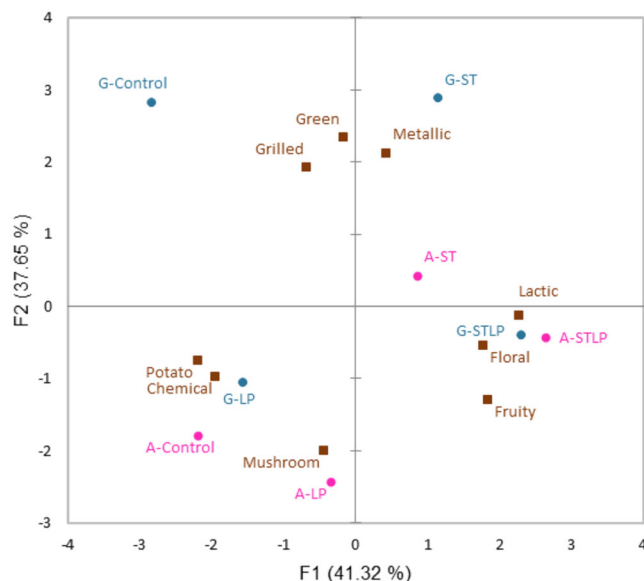


FIGURE 3 Principal component analysis (PCA—Pearson correlation) performed on the 9 odor classes (■) of pea protein albumin (A) and globulin (G) fractions (●) obtained without (Control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP). The volatile compounds with DF (detection frequency) ≥ 4 assessors were considered.

3-methylbutan-1-ol (metallic), 3-hydroxybutan-2-one (lactic), 2,5-dimethylpyrazine (grilled), (*Z*)-non-2-enal (green), (*E*)-non-2-enal (green), geraniol (fruity), 2-phenylethanol (floral), γ -octalactone (fruity), γ -decalactone (fruity), δ -decalactone (fruity), 2-aminoacetophenone (floral), δ -jasmin lactone (fruity), and methyl dihydrojasmonate (floral). Of these, only butane-2,3-dione (lactic) and 3-methylbutan-1-ol (metallic) were detected in A-STLP, γ -octalactone (fruity) in G-STLP, 2,5-dimethylpyrazine (grilled) in G-ST, (*Z*)-non-2-enal (green) in A-ST, and α -terpineol (floral) and geraniol (fruity) in A-LP. 4,5-Epoxy-(*E*)-dec-2-enal (metallic) and oct-1-en-3-one (mushroom) had higher DF in G-LP, and they were detected by all the assessors in this sample. Only geranylacetone (chemical) was detected in all the albumin fraction samples (fermented and control).

PCA was performed on the average of DFs (≥ 4 assessors) of the 9 odor classes defined in the samples (Figure 3). The first and second components accounted for 78.97% of the total variance. The first component axis separated the samples fermented with ST and STLP from the others. Lactic, floral, and fruity notes were mainly attributed to A-STLP, G-STLP, and A-ST. On the opposite, G-LP, A-LP, G-Control, and A-Control were classified by potato, chemical, and mushroom attributes. The second component axis separated G-ST and G-Control from the other samples for having higher values in green, grilled, and metallic notes. The dominant grilled note in G-ST is proba-

bly related to the 2,5-dimethylpyrazine (grilled), which was detected only in this sample.

To better understand the differences between the samples, PCA was performed for the albumin and globulin fractions separately. The first and second components for albumin fractions (Figure 4a) accounted for 65.38% and 29.60%, respectively. The first component separated A-STLP and A-ST from A-LP and A-Control. A-STLP was described mainly by fruity, floral, lactic, and metallic notes. The metallic odor attribute in A-STLP was assigned to the presence of “3-methylbutan-1-ol,” which was detected only in this sample (Table 2). A-ST is defined mainly by green attributes. The green odor attribute could originate mainly from the presence of (*Z*)-non-2-enal, which was detected only in this sample (Table 2). A-LP and A-Control fractions were characterized mostly by chemical, potato, and mushroom notes.

In globulin fractions (Figure 4b), the first and second components accounted for 52.47% and 30.93%, respectively. The first component separated G-Control from fermented samples. G-STLP was mainly characterized by floral, fruity, and lactic notes. Floral and lactic seemed to be common in G-ST and G-STLP. However, G-ST had green, metallic, and grilled notes, unlike G-STLP.

4 | DISCUSSION

In this study, the aromatic profile of pea albumin and globulin fractions obtained from the lactic acid fermentation or conventional acidification with hydrochloric acid toward the isoelectric point of pea proteins were characterized by GC-MS and GC-O. The formation and evolution of volatile compounds in peas as well as the effect of fermentation on the recovered fractions are further discussed.

The majority of the volatile compounds identified in this study originated from lipid degradation in all the samples. Among samples, G-Control had the highest content of volatile compounds originating from fatty acids, such as pent-1-en-3-ol, pentan-1-ol, oct-1-en-3-ol, (3*E*,5*E*)-octa-3,5-dien-2-one, and pentane-2,3-dione. The presence of these compounds has been reported previously for unfermented pea protein products (Ben-Harb et al., 2020; Kaczmarek et al., 2018; Murat et al., 2013). They are responsible mainly for the so-called beany note in pea protein (Fischer et al., 2022b). However, it is important to consider that the development of beany note depends on the concentration of the responsible volatile compound (Vara-Ubol et al., 2004). Also, the mixture of different volatile compounds at various concentrations could alter the perception of certain notes (Bott & Chambers IV, 2006). For instance, “hexanal” has no beany odor, but its mixture with a beany volatile could increase the intensity of the beany odor. In general,

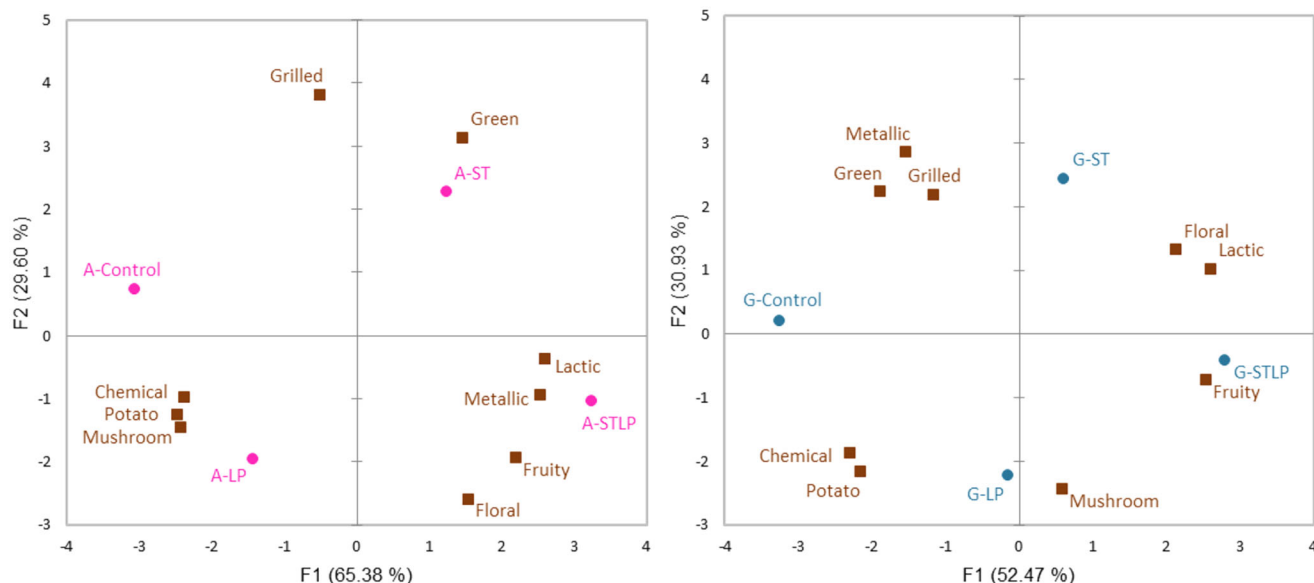


FIGURE 4 Principal component analysis (PCA—Pearson correlation) performed on the 10 odor classes (■) of pea protein albumin (A) (a) and globulin (G) (b) fractions (●) obtained without (Control) or with fermentation by *S. thermophilus* (ST) and *L. plantarum* (LP) or their coculture (STLP). The volatile compounds with DF (detection frequency) of 45% and higher were considered.

aldehydes are the main compounds responsible for pea protein off-flavors, and they were present mainly in globulin fractions and in particular G-Control, which had the highest value in tetradecanal, 2-ethylhexanal, octanal, and hexanal. This could be explained by the type of protein and their affinity to the volatile compounds in globulin fraction, as changes in protein structure and concentration could alter their flavor-binding affinities (Guichard, 2002). Indeed, these volatile compounds are nonpolar ($\log p > 1$), and they have low solubility in water. Hence, they would be retained strongly by the hydrophobic structure of globulin and enriched this fraction during protein precipitation (Heng, 2005). This could be the reason why A-Control had a lower content of volatile compounds compared with G-Control. In contrast to G-Control, a higher content of acids, in particular pentanoic acid, was observed in A-Control, which could be explained by the higher solubility of these compounds and its higher distribution in the soluble fraction.

The volatile compounds responsible for off-flavors were significantly reduced after fermentation in albumin and globulin fractions, which could be related to the enzymatic activity of LAB. A good example of the enzymatic activity of LAB could be converting the aliphatic aldehydes either to alcohols by alcohol dehydrogenase (ADH) or to carboxylic acids by aldehyde dehydrogenase (ALDH) enzymes (Fischer et al., 2022b). For instance, hexanal and octanal, with high concentrations in G-Control, were converted probably to hexanoic acid in A-ST, octanoic acid in A-ST, A-STLP, and A-LP, respectively, through the activity of ALDH. Ben-Harb et al. (2019) reported the reduction

of aliphatic aldehydes after fermentation of pea protein products with *L. plantarum*. In fermented samples, LP and STLP had a lower content of aldehydes compared with ST. The differences might be explained by the heterofermentative nature of LP compared with homofermentative ST, as Harlé et al. (2020) suggested that the heterofermentative LAB strains showed a higher ability to reduce aldehydes than homofermentative strains (ST). It was reported previously that heterofermentative strains have more ADH and ALDH compared with homofermentative strains (Fischer et al., 2022b).

Some ketones responsible for green notes, such as pentane-2,3-dione and (3*E*,5*E*)-octa-3,5-dien-2-one (Ebert et al., 2022; Shi et al., 2021), were not detected in albumin-fermented or decreased in globulin-fermented fractions comparing to control. However, the content of some other ketones such as heptan-2-one, 3-hydroxybutan-2-one, 1-hydroxypropan-2-one, decan-2-one, and butane-2,3-dione were increased in fermented samples. The increase observed in the content of some volatile compounds after fermentation could be explained by the formation of volatile compounds during the metabolic pathways of bacteria, that is, glycolysis, proteolysis, and lipolysis (Emkani et al., 2022). Lactate is the main product of sugar metabolism (glycolysis), which can be alternatively converted into different volatile compounds. For instance, butane-2,3-dione, also known as diacetyl, was an important product of the glycolysis metabolism of LAB (Cogan & Hill, 1993). The highest concentration of this compound belonged to the samples fermented with STLP, which presented the highest rate of acidification (Emkani et al.,

2023). It is worth noting that butane-2,3-dione with a lactic note was detected only in A-STLP in an analysis of GC-O. The increase in the content of butane-2,3-dione after fermentation has been already reported for fermented pea protein products with LAB strains (Ben-Harb et al., 2020); for lupin flour with commercial yogurt culture (*Lactobacillus delbrueckii* and *S. thermophilus*) (Kaczmarek et al., 2018); for pea protein isolate with different LAB, including *L. plantarum* (Arteaga et al., 2021). Harlé et al. (2020) reported that in the fermentation of soy with different LAB, butane-2,3-dione was produced mainly by *S. thermophilus* strains. 3-Hydroxybutan-2-one, known as acetoin, is another metabolite of glycolysis that was found in A-ST, A-STLP, and G-STLP with high content. Mefleh et al. (2022) observed that 3-hydroxybutan-2-one had higher content in samples fermented with *S. thermophilus* monoculture and its coculture with *L. plantarum* compared with unfermented and the ones fermented with coculture of *S. thermophilus* and *L. lactis*. Harlé et al. (2020) have also reported the high content of 3-hydroxybutan-2-one for fermented soy with different strains of *S. thermophilus* and *L. plantarum*.

Proteolysis is another important metabolic activity of LAB. The respective activity of proteases, amino carboxypeptidases, and decarboxylases produces different peptides, amino acids, and α -keto-acids, which can be converted to various volatile compounds, including, aldehydes, acids, esters, alcohols, and pyrazines (Ben-Harb et al., 2019). For instance, 3-methylbutan-1-ol and 2-methylpropanol originated from the metabolism of the branched amino acids, leucine and isoleucine (Morales et al., 2003). 3-Methylbutan-1-ol had the highest value in albumin-fermented samples compared with all the samples with any difference between globulin-fermented samples and globulin control. This compound was not detected in A-Control. This represented the hydrolysis of branched-chain amino acids during fermentation, which were then the precursors of volatile compounds with lower partition coefficient ($\log P < 1$) and they had fewer affinities with hydrophobic sites of the protein; hence, they were found in the soluble fraction (albumin) (Heng, 2005). High proteolytic activity of the bacteria used in the present study, especially in coculture (STLP), and the production of small peptides and amino acids in fermented pea protein fractions has already been shown (Emkani et al., 2023). Indeed, 3-methylbutan-1-ol with metallic note was detected only in A-STLP in analysis by GC-O. This volatile compound is responsible for the malty aroma in cheese, and it can be formed during the enzymatic activity of certain LAB (Meng et al., 2021). Here it was classified in the metallic odor class because of the odor attributes described by some assessors. Benzenemethanol was derived from the degradation of phenylalanine. It had the

highest content on A-STLP and A-LP and followed by A-ST. In GC-O analysis, benzenemethanol with a fruity note was also detected in all the fermented albumin fractions, as well as G-STLP and A-Control. Benzaldehyde, which was also obtained from the degradation of phenylalanine, had the highest value in G-STLP and G-ST. Furthermore, fermented samples had a higher content of acids. Acids are the main products of the enzymatic activity in LAB. Acetic acid and 2-methylbutanoic acid originated from amino acids (Nakae & Elliott, 1965). Acetic acid originated from alanine and it had a more eminent value in A-ST compared with all the other samples. The lowest value of this compound was found in G-Control, G-ST, and G-LP. However, this compound was not detected in GC-O analysis for any sample. However, 2-methylbutanoic acid originated from isoleucine, and it had the highest value in A-LP. The high concentration of these acids in fermented samples could indicate the high proteolytic activity of these strains. The high content of acid acetic in ST could also be related to the homofermentative nature of this strain. In fact, homofermentative LAB produces lactic acid as a sole product of hexose fermentation. However, at slow growth or substrate limitation, homofermentative strains would change to mixed acid fermentation in which they produce lactic acid, acetic acid, and formic acid (Nuryana et al., 2019). It has been previously observed that it took longer for ST to reach pH 4.8 compared with LP and STLP (Emkani et al., 2023).

The majority of LAB have lipase and esterase activity. The activity of lipase during fermentation produces various volatile compounds such as lactones, esters, acids, and alcohols. γ - and δ -lactones are produced by β -oxidation of unsaturated fatty acids (Chen et al., 2017). The contents of lactones were higher in fermented samples, especially the ones fermented with the coculture (STLP). There is evidence that some LABs have high activity toward hydroxylation of fatty acids, which are subsequently converted to the corresponding γ - or δ -lactones (Suzuki et al., 2016). Probably higher enzymatic activity of STLP toward the generation of lactones could be related to the synergetic effect of the bacteria. Esters, which contribute to the floral and fruity notes of the products, are formed by the esterification of an alcohol with an organic acid through the metabolism of protein, sugar, and fat. For instance, ethyl butanoate had a higher concentration in fermented albumin samples compared with the other samples, and the highest value belonged to A-ST. Liu et al. (1998) reported a noticeable production of ethyl butanoate in dairy products fermented with *S. thermophilus* strains. This volatile compound is formed by esterification of ethanol with butyric acid (Liu et al., 1998). The intracellular esterase of LAB is able to synthesize or hydrolysis esters. The highest concentration of esters in analysis of GC-MS mainly

belonged to A-ST and A-STLP. In GC-O analysis also, esters were detected mainly for the samples obtained by ST and STLP.

It is important to note that not all the OACs detected by GC-O were identified by GC-MS. As indicated before, seven of them were not detected by GC-MS, which means that the intense odor-active zones are not necessarily correlated with the highest peak signals. In other words, volatile compounds with trace quantity could be an important aroma contributor while one with higher quantity may not have a direct impact on aroma perception (Table 1). For instance, 2-isobutyl-3-methoxypyrazine was not detected in GC-MS, but it was perceived in GC-O with its distinctive “bell pepper” odor. Also, the LRI values were in agreement with those found in the database and those obtained by injecting pure molecules. Indeed, pyrazines with their low olfactory threshold (0.001–0.002 g/L in water) contributed significantly to the aroma profile of pea protein. However, the low concentration of pyrazines in pea protein makes it difficult to extract these compounds sufficiently for detection (Benavides-Paz et al., 2022).

The analysis of GC-O permitted us to better distinguish the aromatic traits between the samples. For instance, G-Control and A-Control were mainly characterized by chemical, metallic, mushroom, green and grilled. Furthermore, samples fermented with LP had more similar characteristics to samples obtained by control. This is probably for the presence of volatiles, such as methional (potato), 2,6-dimethoxyphenol (chemical), (*Z*)-hex-3-en-1-ol (metallic), (*Z*)-non-2-enal (green), geranylacetone (chemical), and oct-1-en-3-one (mushroom). Indeed, the mushroom odor corresponding to oct-1-en-3-one and oct-3-en-2-one had a noticeable DF in all the samples. Schindler et al. (2011) reported the presence of these two compounds for lupin protein fermented with *L. plantarum*. Schindler et al. (2012) also detected oct-1-en-3-one in both fermented and unfermented pea protein. However, oct-3-en-2-one in their study was detected only in fermented pea protein (Schindler et al., 2012). This could suggest that the fermentation has almost no effect on the reduction of these compounds.

The samples fermented by STLP and ST were clearly characterized by lactic, floral, and fruity odor. Here, the fruity perception in fermented samples corresponded mainly to lactones, esters, and alcohols as observed in GC-MS. Generally, lactones have low perception thresholds in pea protein (Karolkowski et al., 2021). However, the production of these compounds with LAB would increase their perception (Suzuki et al., 2016). Here, δ -decalactone (fruity) was detected only in all the fermented samples, γ -decalactone (fruity) was perceived only in A-STLP and G-STLP, γ -octalactone only in G-STLP, and jasmin lactone

(fruity) only in A-LP, A-ST, and G-STLP. It seems γ -lactones (i.e., γ -octalactone, γ -nonanolactone, and γ -decalactone) have the highest detection percentage in the fermented globulin fractions. However, δ -lactone (i.e., δ -decalactone and δ -Jasmine lactone) has a higher percentage in fermented albumin fractions. Although their log *P* is very close, they do not share similarities between two fractions of albumin and globulin. Methyl dihydrojasmonate with floral note was not detected in control fractions, and it had the highest detection in samples fermented with ST, STLP, and A-LP. Ethyl-3-methylbutanoate or ethyl-2-methylbutanoate with fruity note was detected only in A-STLP. This compound might have been originated from the esterification of 3-methylbutan-1-ol (Murray et al., 1976). Lactic attribute in ST and STLP samples was related to the presence of 3-hydroxybutan-2-one (lactic). Also, butane-2,3-dione (lactic) was detected only in A-STLP as found by GC-MS.

In addition, some OACs, known to be pea protein off-flavors, such as (2*E*,4*E*)-nona-2,4-dienal (grilled), (2*E*,4*E*)-deca-2,4-dienal (grilled), 2,6-dimethoxyphenol (chemical), 2,5-dimethylpyrazine (grilled), (*Z*)-hex-3-en-1-ol (metallic), and 6-methylheptan-2-one (chemical), were not detected in STLP samples. Probably the absence of (2*E*,4*E*)-deca-2,4-dienal (grilled) and (2*E*,4*E*)-nona-2,4-dienal (grilled) in samples fermented with STLP could be related to the double-bond hydration and retro-aldol degradations of these compounds, which yielded other aldehydes such as (*E*)-oct-2-enal (green) (Josephson & Lindsay, 1987) or degraded to other nonvolatile compounds by microbial metabolism. All these considered, it could be said that the odor of the samples fermented with STLP could be more pleasant because they had a higher intensity of positive attributes compared with ST and LP alone. This might be for the synergetic effect of the bacteria that would favor the formation of volatile compounds in the samples obtained by coculture, which were not detected in monocultures such as 2-methylpropan-1-ol (fruity), butane-2,3-dione (lactic), and γ -octalactone (fruity). Madjirebaye et al. (2022) reported excellent sensorial properties for soymilk fermented with coculture of *S. thermophilus* and *L. plantarum* rather than *S. thermophilus* and *L. plantarum* alone. Mefleh et al. (2022) observed a higher score of sweet and creamy attribute for the chickpea fermented with coculture of *S. thermophilus* and *L. plantarum* compared with *S. thermophilus*.

5 | CONCLUSION

To conclude, a high concentration of some pea off-flavors was observed in the aroma profile of pea protein fractions obtained by traditional acidification by adding hydrochloric

ric acid (control) during extraction (such as (2*E*,4*E*)-deca-2,4-dienal (grilled) and (2*E*,4*E*)-nona-2,4-dienal (grilled)). These compounds were more retained in the globulin fraction compared with the albumin fraction, probably owing to the hydrophobicity of globulins and the nonpolar nature of these volatile compounds. The quantity of these volatile compounds was reduced after fermentation through the enzymatic activity of LAB. Furthermore, the quantity of some other volatiles with positive perceptions (such as 2-methylpropan-1-ol (fruity)) increased through the metabolic pathway of LAB. Albumin-fermented samples presented a higher content of these positive volatile compounds compared with globulin-fermented samples, probably due to increased solubility ($\log P < 1$) of the aroma compounds. The samples obtained from coculture fermentation (STLP) were described by fruity, floral, and lactic attributes. Clearly, the synergies between ST and LP permitted us to obtain positive notes related to the presence of compounds such as γ -octalactone (fruity), γ -decalactone (fruity), butane-2,3-dione (lactic), and 2-methylpropan-1-ol (fruity), which were not observed in samples obtained by monoculture fermentation. Changes in volatile profile are clearly strain dependent. As a result, the aromatic profile of pea proteins can be shaped through the meticulous selection of suitable fermenting strains. These findings could be complemented by sensory analysis to obtain a comprehensive understanding of the protein systems.

AUTHOR CONTRIBUTIONS

Mehrsa Emkani: Writing - Original Draft; Writing - Review & Editing; Investigation; Formal analysis; Validation; Methodology. **Karine Gourrat:** Writing - Review & Editing; Investigation; Validation; Methodology. **Bonastre Oliete:** Supervision; Writing - Review & Editing; Conceptualization; Methodology. **Rémi Saurel:** Supervision; Funding acquisition; Project administration; Writing - Review & Editing; Conceptualization; Methodology.

ACKNOWLEDGMENTS

This work was supported by the French Agence Nationale de la Recherche (ANR PEAVALEU project, grant ANR-19-CE21-0008-03) and by the European Regional Development Fund (FEDER-FSE Bourgogne 2014/2020, grant No. BG0025913). The authors would like to thank the ChemoSens Facility of INRAE Dijon for the access to the equipment. The authors would also like to thank Christophe Martin for his valuable advice in statistical analysis and Sylvie Moundaga for her invaluable support and guidance in bacterial preparations.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The original contributions are presented in this study, and further inquiries can be directed to corresponding author.

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How to cite this article: Emkani, M., Gourrat, K., Oliete, B., & Saurel, R. (2024). Identification of volatile and odor-active compounds in pea protein fractions obtained by a modified extraction method using fermentation. *Journal of Food Science*, 1–21. <https://doi.org/10.1111/1750-3841.17145>