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Supercritical Carbon Dioxide + Ethanol Extraction to Improve Organoleptic Attributes of Pea Flour with Applications of Sensory Evaluation, HS-SPME-GC, and GC-Olfactory

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Abstract: Supercritical carbon dioxide + ethanol (SC-CO_2+EtOH) extraction, was employed as a deflavoring method to improve the sensory properties of pea flours. Furthermore, the impacts of particle size along with extraction on volatile profile and sensory attributes of pea flours were investigated using multiple approaches. These included headspace solid-phase microextraction-gas chromatography (HS-SPME-GC), GC-olfactometry (GC-O), and quantitative descriptive analysis (QDA) using a trained sensory panel. Total volatile contents of non-deflavored and deflavored whole pea flour and its fractions were in the range of 7.1 ± 0.3 to 18.1 ± 1.0 μg/g and 0.4 ± 0.1 to 2.7 ± 0.4 μg/g, respectively. The GC-O system showed that the total volatile intensity was in the range of 14.5 to 22.0 and 0 to 3.5, for non-deflavored and deflavored pea flours, respectively. Volatile analyses indicated that 1-hexanol, 1-octanol, 1-nonanol, nonanal, and 2-alkyl methoxypyrazines were major off-aroma compounds. Most off-aroma compounds were not detected in deflavored pea flours. QDA revealed less pea intensity and bitterness of deflavored pea flours. The larger particle size of flours resulted in less off-aroma compounds based on the GC data but more bitterness based on QDA. The SC-CO_2+EtOH extraction at optimum conditions and particle size modifications can be a potential technology to improve the organoleptic properties of pulse ingredients.

Keywords: *Pisum sativum*; off-flavor; supercritical carbon dioxide; pea intensity; saponins; pea flour; pea protein; chemometrics

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1. Introduction

As global awareness for healthy lifestyles increases, there has been an increasing demand for healthier (e.g., high fiber), nutritious plant-based (e.g., high protein and fiber), and gluten-free foods [1,2]. The rapid growth in the human population, expecting to reach 9.5 billion by 2050, combined with increased disposable income are drivers for growing consumer demands for nutrient-dense foods [1–3]. Addressing these demands has created challenges for food scientists to reformulate food products with sustainable, nutritious food ingredients, which have acceptable sensory quality [4].

The flour prepared from dry pea (*Pisum sativum* L.) is an attractive gluten-free and non-GMO food ingredient that has an outstanding nutritional profile (e.g., high protein, good complex carbohydrates, high folate, and micronutrient contents) and potential health benefits [5–7]. However, pea flour, similar to other pulse flours, has been underutilized due to its undesirable flavor or off-flavor usually described as "beany", "pea", "earthy", "green" and "bitter" [8–10]. This off-flavor limits the potential utilization of pea ingredients in the food system and mitigates their market value [4,11].

The off-flavor of dry peas can be either present naturally or developed during harvesting, processing, and storage [12–14]. The pea off-flavor is the combination of off-aroma

compounds that are volatile organic compounds (VOCs), which impart strong pea aroma, and off-taste compounds that are non-VOCs, causing bitterness [10,11]. Different VOCs identified in dry peas can develop through lipid oxidation and amino acid degradation pathways [10,11,15]. The degradation of pea lipids and unsaturated fatty acids (e.g., linoleic acid) through enzymatic (i.e., hydrolytic and oxidative processes) and non-enzymatic (i.e., autoxidation) reactions generates significant amounts of alcohols, aldehydes, ketones, and furans, causing off-flavor in dry peas [12–14]. Lipase hydrolyzes lipids to free fatty acids, which are then oxidized by lipoxygenase (LOX) and autoxidation pathways [12]. The secondary products of lipid oxidation possess a distinct undesirable aroma. Alcohols, such as 1-hexanol, contribute a green aroma while grassy and citrus odor are caused by the aldehydes, hexanal, and nonanal, respectively [8,10]. Alkyl methoxypyrazines, 3-*sec*-butyl-2-methoxypyrazine, and 3-isobuthyl-2-methoxypyrazine, are produced from amino acids in the seed [16]. These methoxypyrazines are important contributors to "perceived green pea aroma" with extremely low olfactory thresholds [14,16,17]. The bitterness of peas is associated with non-VOCs, saponins, contributing to the off-taste development [18].

The renewed interest in supercritical carbon dioxide (SC-CO_2) extraction is based on a desire by the food industry to use a sustainable green technology for the extraction of natural substances from plant materials compared to conventional extraction systems [19–21]. The separation of various natural substances, including flavor and fragrances (e.g., essential oil), aroma extracts from fruit, spices, and herbs, natural antioxidants and food colors (e.g., carotenoids), plant and animal lipids, and volatile compounds from plant materials have been widely reported in the literature [10,21–25]. Furthermore, the SC-CO_2 extraction has a physical approach to modify starch and starch gelatinization properties [26–29]. Carbon dioxide (CO_2) is the most popular supercritical fluid due to its low cost, availability, non-flammability, and non-toxic nature (food grade). Furthermore, CO_2 moderate critical conditions (temperature, 31.1 °C; pressure, 7.4 MPa) minimize damage to plant materials [25,30]. Moreover, the uniqueness of SC-CO_2 extraction is the speed at which physical parameters (e.g., pressure, temperature, co-solvent) and polarity of the extractant can be adjusted. This allows for the separation of moderately polar (e.g., aldehydes, ketones, and esters) and non-polar compounds (e.g., alkenes and terpenes) in a short time with less energy requirement. Furthermore, this extraction system can be assisted with a polar co-solvent (e.g., ethanol, methanol) to enhance the solubility of SC-CO_2 for the extraction of polar organic compounds (e.g., alcohols, saponins, xanthophylls, phenolics). Ethanol (EtOH) has been favored as a co-solvent to separate polar compounds [10,24,31].

The optimized SC-CO_2+EtOH extraction was successfully employed to improve the organoleptic attributes of yellow pea flour through the removal of off-flavor compounds [10]. Flavor modification of pea flour using the SC-CO_2+EtOH extraction at optimum conditions was conducted in less time and using less ethanol [10] compared to other conventional solvent-based deflavoring methods for pea ingredients [32,33]. Furthermore, the efficiency of this extraction for flavor modification of pea ingredients might be more promising than bio-processing approaches, such as fermentation [34] and germination [15], which contributed to the formation of new VOCs, such as ester formation.

Significant effects of particle size on the physicochemical and functional properties of pea flour have been previously reported [20,35]. However, impacts of particle size on the volatile profile and sensory properties of pea flours have not been examined. Therefore, the objective of the present study was to assess the applicability of SC-CO_2+EtOH extraction for deflavoring pea flour with different particle sizes and to determine the interaction effect between the two factors (i.e., SC-CO_2+EtOH and particle size) on the volatile profile and sensory quality of yellow pea flours. For this purpose, instrumental analyses, headspace-solid phase microextraction-gas chromatography (HS-SPME-GC), and GC-olfactory (GC-O) detection along with the quantitative descriptive analysis (QDA) as a sensory evaluation technique were applied to determine changes in selected off-aroma compounds and sensory attributes in pea flours.

2. Materials and Methods

2.1. Materials

Viterra (Minot, ND, USA), Specialty Commodities (Fargo, ND, USA), and SK Foods (Moorhead, MN, USA) were the source of whole yellow pea used in this study. The three sources of pea were blended in equal parts (50 kg) using a paddle mixer located at the Northern Crops Institute (Fargo, ND, USA) to create a composite sample (150 kg). The blended samples were hammer milled (Fitzpatrick, Elmhurst, IL, USA) using a 1.270 mm screen and hammer rotation of 102 m/s (7200 rpm). The milled whole pea flour was stored in sealed polyethylene bags at 20 °C until required for deflavoring. Information on the carbon dioxide and ethanol used for extraction, VOCs used to make standard curves, and sensory supplies can be found in a previous publication [10].

2.2. Particle Size Determination

The particle size separation of yellow pea flour obtained from hammer milling was performed using a Ro-tap (W.S. Tyler, Mentor, OH, USA) with a series of sieves having openings of 425 (40-mesh), 250 (60-mesh), 150 (100-mesh), 106 (140-mesh), and 53 (270-mesh) µm based on the approved method 55-60.01 [36]. The particle sizes obtained were classified as fractions, consisting of several particles: 425 µm $\geq$ flour > 250 µm (coarse/large), 250 µm $\geq$ flour > 150 µm (medium), and 150 µm $\geq$ flour > 106 µm (fine/small). Unseived yellow pea flour (hereafter referred to as whole flour since all particles were present in this flour) was used for further analyses along with coarse, medium, and fine fractions. Further information based on the particle size distribution of pea flour fractions was previously published [20].

2.3. SC-CO_2+EtOH Extraction

The three fractions and whole yellow pea flour were subjected to SC-CO_2+EtOH extraction, separately, using the optimum deflavoring conditions (22% ethanol, 86 °C, and 42.71 MPa) described by Vatansever and Hall [10] without modification. Briefly, a laboratory scale ISCO supercritical fluid extractor (Model SFX 2-10; Isco, Inc., Lincoln, NE, USA) was used with the main solvent, CO_2 (99.99% purity), and co-solvent, ethanol (200 proof, undenatured). In the system, ethanol (22%) was pumped continuously into CO_2. The raw pea flour (6 g) was placed in stainless steel vials with frits. The extraction was employed at 22% ethanol, 86 °C, and 42.71 MPa. The raw pea flour was subjected to a 40-min total extraction, including a 10-min static and a 30-min dynamic extraction at a flow rate of 2 mL/min. Then, the ethanol residue of deflavored flour was removed by drying the flour at 70 °C in a convection oven for 1 h. The deflavored pea flours were stored in 2.5 mil Mylar bags (Uline; Pleasant Prairie, WI, USA) at −20 °C until needed.

2.4. Headspace Solid-Phase Microextraction-Gas Chromatography (HS-SPME-GC) Analysis of Selected Volatile Compounds

The volatile detection of non-deflavored and deflavored pea flours (whole, coarse, medium, and fine) was measured using HS-SPME-GC (Agilent 7820A, Agilent Technologies, Inc., Santa Clara, CA, USA) with FID following the protocol described by Hall et al. [37] with some modifications. Briefly, 1 g of pea flour was added to a 4 mL vial and sealed using PTFE silicone Septa (Supelco, Bellefonte, PA, USA). The sample was heated in a 95 °C water bath for 10 min. The SPME filament (DVB/CAR/PDMS, 50/30 µm; Supelco, 57328-U, Bellefonte, PA, USA) was inserted for 15 min while the sample was heated at 90 °C. Then, the filament was transferred to the injection port of the GC and remained to desorb for 7 min. The volatile analysis was performed under the following conditions: Helium flow rate of 33.7 mL/min, initial oven temperature of 35 °C, and ramped to 180 °C at 10 °C/min, then, maintained for 12 min at 180 °C.

Each VOC was identified by comparing the retention time of the chosen standards (as seen in Appendix A Figures A1 and A2) and quantified (µg/g) using the standard curve (Appendix A Table A1). Then, the total volatile (TV) concentration in pea flour was obtained

from the sum of VOCs (μg/g), which were selected based on previous studies [8,12,17]. A standard curve was constructed in a solid matrix (i.e., finely ground fresh saltine cracker) through the dilution of the standards based on the protocol described by Vatansever and Hall [10] without any modifications. The R^2 values of the chosen standards (Appendix A Table A1) had a range of 0.9746 to 0.9990.

2.5. Gas Chromatography-Olfactory (GC-O) Training

A specific GC-O training composed of vocabulary, reference mixture, and real sample training with five healthy, nonsmoking trainees (one male and four females) were completed based on the protocols of Vene et al. [38] with some modifications. These trainees were informed before the analysis to abstain from alcoholic drinks, spicy meals, and other strong flavorful foods. Additionally, the trainees did not have access to chromatogram results and did not communicate with one another during testing to produce reliable results.

2.5.1. Vocabulary Training

The vocabulary training reported by Vene et al. [38] took place using fourteen standard aroma compounds, which were used for the standard curve preparation [10]. The stock solutions, which were 2800 mg/L for each standard except for γ-valerolactone, which was 3500 mg/L, were diluted with methanol to prepare 1000 and 500 mg/L for each standard. Then, the samples were prepared using sniffing strips (1 cm) dipped into the solutions (i.e., 500, 1000, and 2800 or 3500 mg/L for the fourteen compounds). After the removal of methanol residue, the strips were placed into screw-cap tubes (20 mL). The training section was arranged as a group discussion. The trainees smelled the solution in the vials, including sniffing strips, to determine the experimental descriptor and also to assess the degree of intensity of each standard for three concentrations using a five-point scale, where 1: Very weak, not identifiable; 2: Weak, but identifiable; 3: Moderate; 4: Strong; and 5: Highly strong. After vocabulary training, the trainees continued sniffing the standards for 4 weeks to memorize each VOC.

2.5.2. Reference Mixture Training

Seven randomly selected standards were used to make a reference mixture for the training base on methods described by Vene et al. [38] and Xu et al. [15] with some modifications. The reference mixture consisted of 0.1 mL of each of the seven randomly selected standards that resulted in 0.7 mL total volume. Considering that the volatiles selected have different detection thresholds, normalization of the reference mixture was done by selecting standard concentrations (0.14 to 0.5 mg/mL) based on the intensity rating by the trainees during vocabulary development. All the standards used in the reference mixture were rated as moderate intensity (3.0 to 3.5) by the trainees. Overall, two reference mixtures were prepared using fourteen selected standards and used to train panelists on the GC-O protocol. The data produced by each trainee was evaluated based on detected peaks.

2.5.3. Real Sample Training Using Pea Flour

In the last training session, pea flour composed of the target and other compounds were used to train the trainees for the real sample training based on the method described by Xu et al. [15]. The odor intensity of a VOC was recorded using a posterior intensity method. Through this method, the intensity of the recognized compound was identified and compared with the mass spectrum obtained from GC/MS to evaluate the results of each trainee. After conducting each training session, the trainees were informed on their results and comments were provided [15].

2.6. Headspace Solid-Phase Gas Chromatography Mass Spectrometry Olfactory (HS-SPME-GC/MS-O) Analysis

Three batches of each flour treatment (i.e., after SC-CO_2+EtOH extraction) were blended to create a homogeneous sample for this analysis. Briefly, blended pea flour (2 g) was placed in 20 mL vials and sealed with a screw cap with PTFE silicone Septa (Supelco, Bellefonte, PA, USA) and transferred to the Agilent Technologies 7890B GC system with a ZB-Wax column (60 m × 0.25 mm and 0.25 µm thickness) using the injection port in splitless mode [15]. The analysis was performed according to Hall et al. [37] with some modifications. The sample was heated for 10 min at 93 °C. The SPME fiber (DVB/CAR/PDMS, 50/30 µm; Supelco, 57328-U, Bellefonte, PA, USA) was placed in the vial for 15 min at 93 °C, and then, inserted into the GC and remained for 5 min to desorb the volatiles. The HS-SPME-GC/MS-O analysis followed these conditions: Helium flow rate of 2 mL/min, initial oven temperature of 35 °C ramped to 180 °C at 10 °C/min then, maintained for 12 min at 180 °C and increased to 200 °C at 9 °C/min and to 250 °C at 45 °C/ml then held for 3 min. The NIST 14 library was used as a database for mass spectrum.

The MS-olfactory analysis was completed based on the protocol described by Xu et al. [15]. Briefly, the column effluent (1/3) was split to the 5977A mass detector and analyzed using the following conditions: Electron impact (EI) ionization port at 70 eV, ion source temperature at 230 °C, scan time segments from 4.00 to 17.89 min, and scanning from m/z 40 to 350. The remaining column effluent (2/3) was split to the olfactory detection port (ODP3; Gerstel, Mulheim an der Ruhr, Germany). The conditions used for the olfactory analysis were as follows: (1) Heating transfer line to the ODP3 at 200 °C; (2) humidifying air of the sniffing port at 30 ml/min; (3) measuring the intensity using a specific remote-control button for quantifying intensity; and (4) recording the experimental descriptor corresponding to each odor using the Gerstel ODP recorder program, including an active microphone to record the data from each panelist when the odor was detected. At the same time, the corresponding peak area to the odors perceived was obtained through the mass spectrum and experimental descriptors of each compound were recorded by each panelist. The peak intensity was measured as the mean of two repetitions for each panelist. Then, all mean intensity scores for each treatment were summed to obtain the total volatile intensity (TVI).

2.7. Sensory Assessment by Quantitative Descriptive Analysis (QDA)

The sensory evaluation of non-deflavored and deflavored pea flours was completed using the QDA technique described by Vatansever and Hall [10] without any modifications. The degree of pea intensity and bitterness of flour samples was measured by eight trained panelists. Each flour sample was replicated three times.

2.8. Statistical Analysis

A two-factor completely random design was used for all analyses. Extraction at two levels and particle size at four levels were the two main factors in this study. The two main factors were considered as fixed effects. A completely randomized 2 × 4 factorial design was used with three replicates, including five and eight observations within each replicate for the HS-SPME-GC and sensory analyses, respectively. The GC-O analysis was completed with this design, including two replicates with five panelists. A Tukey's test at 5% significance level was applied for mean separation. The principal component analysis (PCA) was performed on the mean values of response variables determined by HS-SPME-GC, GC-O, and QDA analyses. The hierarchical cluster analysis (HCA) was applied on the mean values of VOCs determined by HS-SPME-GC and GC-O analyses. All statistical analyses were completed using the JMP software (JMP Pro 15.0.0 version 2019 SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Volatile Compounds Identified in Pea Flour Using the HS-SPME-GC Analysis

The SC-CO_2+EtOH extraction resulted in a significant reduction in total off-aroma compounds determined in pea flours. The TV concentrations (Table 1) of non-deflavored and deflavored pea flours were composed of six alcohols, one aldehyde, two alkyl methoxypyrazines, and one furan (Figure 1). The range of TV concentration was from 7.1 to 18.1 μg/g and 0.4 to 2.7 μg/g for non-deflavored and deflavored pea flours, respectively (Table 1). Significant main and interaction effects ($p < 0.05$) among pea flours were found for the total amount of volatile compounds (Table 2). The TV concentration in deflavored whole yellow pea flour was in agreement with the previous report [10]. In addition to these VOCs, (Z)-3-hexen-1-ol, 2-pentylfuran and γ-valerolactone were determined in non-deflavored pea flours but were not quantified due to their concentrations falling below the lower limit of quantification.

Table 1. Sensory attributes, total volatile, and total volatile intensity (olfactory) of pea flours.

Treatment		Pea intensity	Bitterness	HS-SPME-GC (TV) [A]	GC-O (TVI) [B]
		mm		μg/g	degree of intensity
Non-deflavored	Whole	112.3 ± 4.7 [a]	53.4 ± 4.3 [ab]	18.1 ± 1.0 [a]	19.0 ± 1.5 [a]
	≥250 [C]	106.1 ± 6.4 [ab]	65.5 ± 4.9 [a]	7.7 ± 0.2 [c]	14.5 ± 1.8 [b]
	≥150	87.9 ± 3.6 [b]	38.5 ± 4.8 [bc]	7.1 ± 0.3 [c]	18.0 ± 1.7 [ab]
	≥106	63.0 ± 5.4 [c]	26.7 ± 2.9 [c]	10.3 ± 0.3 [b]	22.0 ± 1.2 [a]
Deflavored	Whole	18.3 ± 3.5 [d]	4.5 ± 1.7 [d]	1.4 ± 0.2 [de]	2.0 ± 0.1 [c]
	≥250	13.0 ± 3.7 [d]	9.7 ± 2.4 [d]	0.4 ± 0.1 [e]	0.0 ± 0.0 [a]
	≥150	12.0 ± 3.5 [d]	8.1 ± 2.8 [d]	0.8 ± 0.1 [e]	2.0 ± 0.9 [c]
	≥106	29.5 ± 5.9 [d]	6.1 ± 2.2 [d]	2.7 ± 0.4 [d]	3.5 ± 1.0 [c]

[A] TV: Total volatile in pea flour detected by HS-SPME-GC. [B] TVI: Total volatile intensity (degree of intensity) in pea flour detected by GC-O. [C] Coarse/large, >250 μm (425 ≥ flour > 250); medium, >150 μm (250 ≥ flour > 150); and fine/small, >106 μm (150 ≥ flour > 106); the whole is unsieved pea flour. Data points were given as mean ± standard deviation. Different letters (i.e., a, b, c, d, e) within columns indicate significant differences ($p < 0.05$) between treatments.

Table 2. F- and *p*-values of main and interaction effects for sensory attributes, total volatile, and total volatile intensity of pea flours [A].

Response Variable	Extraction		Particle Size		Extraction*Particle Size	
	F-Value	*p*-Value	F-Value	*p*-Value	F-Value	*p*-Value
Pea Intensity	491.94	<0.0001	6.84	0.0003	17.94	<0.0001
Bitterness	257.24	<0.0001	13.59	<0.0001	11.17	<0.0001
HS-SPME-GC (TV) [B]	1109.05	<0.0001	91.45	<0.0001	72.19	<0.0001
GC-O (TVI) [C]	622.28	<0.0001	11.67	<0.0001	1.62	0.2112 [ns]

[A] ns: Non-significant at $\alpha = 0.05$ and df =1, 3, 3 for extraction, particle size, and interaction, respectively. [B] HS-SPME-GC (TV): Total volatile by HS-SPME-GC; [C] GC-O (TVI): Total volatile intensity by GC-O.

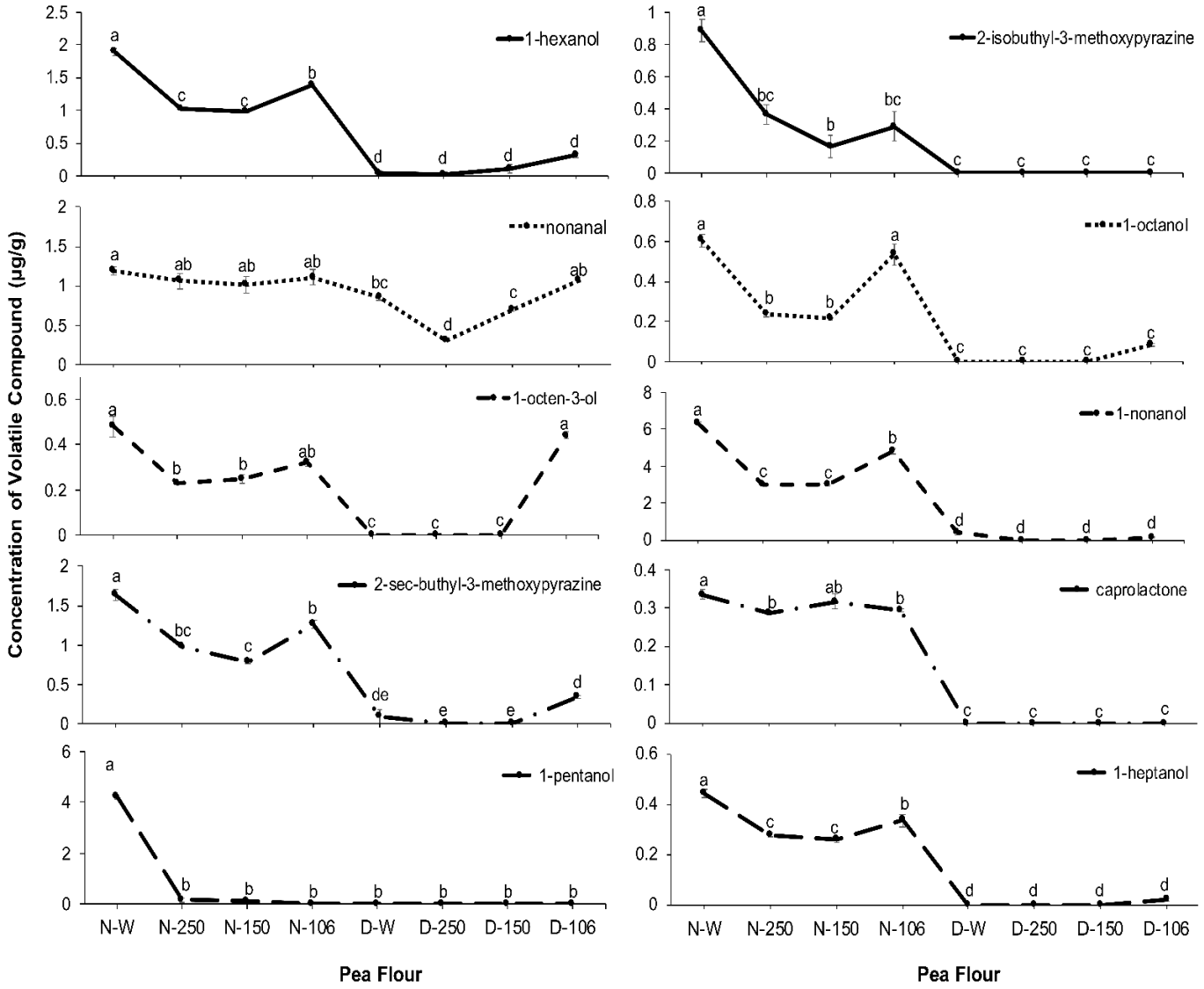


Figure 1. Volatile organic compounds of pea flour detected by headspace solid-phase microextraction-gas chromatography (HS-SPME-GC/FID)z. N-W, N-250, N-150, and N-106 indicates non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively, while D-counterparts are deflavored pea flours. Data points are means ± standard error. Different letters (i.e., a, b, c, d, e) within a volatile compound indicate significant differences ($p < 0.05$) between treatments.

Hexanal, a primary lipid oxidation product of linoleic acid catalyzed by LOX [8], was not detected in pea flours through the HS-SPME-GC system. Similarly, a low hexanal concentration was reported in pea flour [8]. However, Murray et al. [14] observed less hexanal concentration in peas with hexanal: hexanol ratio of 1:200, suggesting that composition of other volatiles might impact hexanal concentration. Murray et al. [14] stated that the less hexanal concentration in peas was when the hexanal:hexanol ratio was 1:200. The inability of the pretreatment (e.g., heating) step of the analytical method to facilitate the release of hexanal from protein may be the reason why hexanal was not found in the pea flours. However, high concentrations of hexanal have been reported in pea protein ingredients [8,33,39], pea protein-based beverages [40], and lentil protein isolate (LPI) [32]. The greater hexanal concentration in pea protein products might be related to the strong aldehyde binding ability of proteins [39]. Anantharamkrishnan et al. [41] recently reported that aroma compounds, such as aldehydes, can form covalent interactions with proteins, which are difficult to cleave and release the aroma compound. Additionally, hexanal can be reduced to 1-hexanol in the presence of alcohol oxidoreductase [14,17]. In whole pea flour, 1-hexanol was quantified as one of the most abundant alcohols after 1-pentanol and

1-nonanol. Likewise, Murat et al. [8] and Wang et al. [33] obtained 1-hexanol as the primary alcohol in pea flour and protein-enriched pea flour (PEPF), respectively.

Of the VOCs, alcohols (e.g., 1-pentanol, 1-hexanol, 1-octanol, and 1-nonanol) were in the highest concentrations in non-deflavored pea flours, ranging from 4.9 to 13.9 μg/g. In contrast, the aldehyde (i.e., nonanal) was the most predominant VOC in deflavored pea flours, ranging from 0.3 to 1.1 μg/g (Figure 1). In the SC-CO_2+EtOH extraction, the addition of ethanol was sufficient to increase the polarity of the system, leading to the removal of alcohols through disruption of hydrogen bonds [10]. Likewise, the greater removal of alcohols, compared to aldehydes, was found in pulse protein ingredients extracted with ethanol [32,33]. The significant removal of 2-*sec*-butyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and γ-caprolactone of pea flours was obtained during the SC-CO_2+EtOH extraction ($p < 0.05$, Figure 1).

The particle size had a significant impact ($p < 0.05$) for the volatile profile of pea flours and also for the efficiency of SC-CO_2+EtOH extraction (Table 2). The selected VOCs, namely 1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol, 1-nonanol, nonanal, 2-*sec*-butyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, and γ-caprolactone, except for 1-pentanol were identified in all non-deflavored pea flours (Figure 1). Previous reports showed similar trends with relatively higher alcohol concentrations in the VOCs [8,39]. Among non-deflavored pea flours, whole pea flour had a significantly ($p < 0.05$) higher TV concentration than other flour fractions (Table 1). Particularly, 1-pentanol, 1-hexanol, 1-nonanol, and alkyl methoxypyrazines were relatively higher in whole pea flour. The fine fraction had greater volatile concentrations than medium and coarse fractions, which may be due to the enhanced removal of VOCs from flour samples with high surface area, such as in the fine particles, during the analytical procedure. Similarly, the enhanced extraction of VOCs from finely ground coffee samples compared to coarse counterparts, having a reduced contact area, was reported by Cordoba et al. [42]. Several VOCs were predominant in pea flour of the fine fraction (Figure 1). These compounds might be embedded in the protein-starch matrix of pea flour and by disruption of this matrix leads to an increased accessibility of these VOCs to extraction. For the finer the particles, the greater disruption of the matrix would be anticipated.

Significant differences ($p < 0.05$) were obtained for the TV concentration in deflavored pea flours, as shown in Table 1. Within these samples, TV was significantly higher in the fine fraction, though the highest TV concentration was found in whole pea flour of non-deflavored samples. This finding indicated that the reduction in particle size decreased the effectiveness of SC-CO_2+EtOH extraction. This was in contrast to what was expected. In theory, finer particles provide a larger surface area that reduces the diffusion path, thus resulting in diminished intra-particle diffusion resistance, and subsequently facilitates the extraction process and efficiency [43]. Ozkal and Yener [30] reported that reducing the particle size of flaxseed resulted in higher oil yield by the SC-CO_2 extraction. However, Khaw et al. [43] showed that excessive particle size reduction caused a decrease in extraction yield likely due to agglomeration, which might lead to CO_2 flows only through micro-channels with a diminished surface area. The SC-CO_2+EtOH extraction removed most VOCs from all pea flours except for the fine fraction owing to a possible agglomeration that caused a reduction in extraction efficiency. Nonanal was the only VOC detected in all deflavored pea flours, which was observed in previous studies [32,33]. Nonanal might be tightly bound in the starch-protein complex via hydrogen bonding or dipole-dipole interactions or covalent interactions, therefore, making it difficult to remove from the starch-protein matrix. A stable binding of aldehydes to proteins through covalent interactions was reported [41].

3.2. HS-SPME-GC/MS-O Analysis of Volatile Compounds in Pea Flours

The TVI values of pea flours based on the GC-O system fell between 0 and 19 (Table 1) and were supported by the changes in selected standard compounds based on processing (Figure 2). The significant main effects ($p < 0.05$) among pea flours were obtained, but

the interaction effect between the two factors was non-significant ($p > 0.05$) for TVI of the selected VOCs (Table 2). Additionally, experimental odor descriptors (e.g., green, lemon, bell pepper, pea, mushroom, sweet) recorded through the GC-O analysis for each VOC (Table 3) agreed with previous reports [8,15].

The SC-CO_2+EtOH extraction significantly ($p < 0.05$) decreased off-aroma compounds in pea flours based on the GC-O results (Figure 2). The TVIs of non-deflavored and deflavored pea flours, containing four alcohols, one aldehyde, two alkyl methoxypyrazines, and one furan, were in the range of 14.5 and 22, and 0 and 3.5, respectively (Table 1, Figure 2). These off-aroma compounds were previously reported through GC-O in pea flours [8,15]. Deflavored pea flours had relatively low TVI, which supported TV results. In comparison to HS-SPME-GC, 1-pentanol and 1-heptanol were not detected by GC-O panelists in non-deflavored pea flours likely due to the concentration present and threshold level. Likewise, Murat et al. [8] reported that 1-heptanol was not detected but 1-pentanol was recorded in pea flour through the GC-O analysis. However, Xu et al. [15] observed the detection of 1-heptanol in germinated pulse flours, unlike non-reporting 1-pentanol in the same samples through the GC-O analysis.

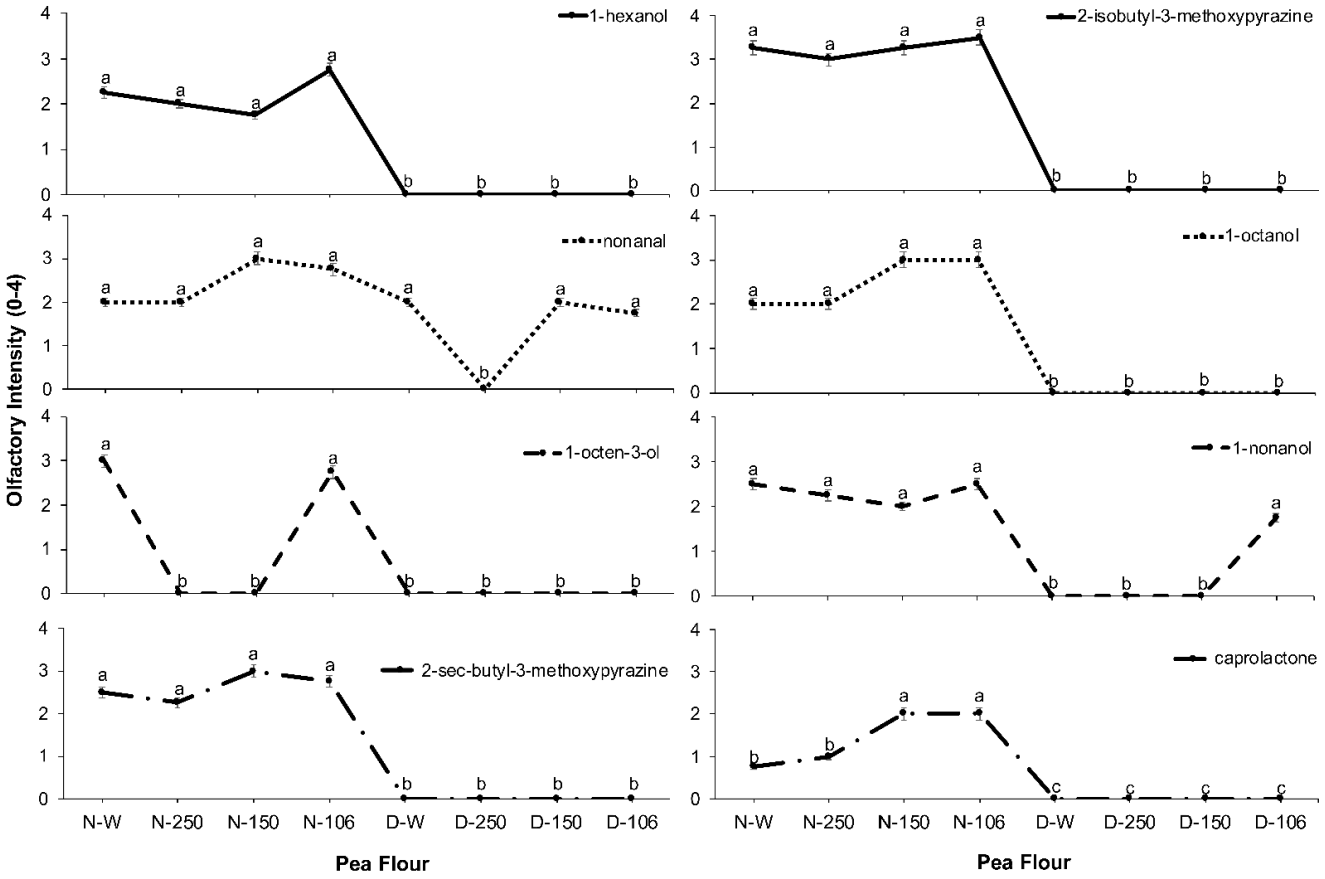


Figure 2. Volatile organic compounds of pea flour detected by the trained panelists of GC-olfactometry (GC-O) analysis. N-W, N-250, N-150, and N-106 indicates non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively, while D-counterparts are deflavored pea flours. Data points are means ± standard error. Different letters (i.e., a, b, c, d, e) within a volatile compound indicate significant differences ($p < 0.05$) between treatments.

Table 3. Aroma compounds identified in the different particle sizes of non-deflavored pea flours by GC-O panelists [A].

Compound	CAS	Theoretical Descriptors	Experimental Descriptors	Origin [B]	N-W [C]	N-250 [D]	N-150 [E]	N-106 [F]
1-Hexanol	928-96-1	Green, hay-like odor	Floral, green, grain, hay-like	Lipid	75%	75%	50%	75%
Nonanal	124-19-6	Waxy, citrus	Lemon, citrus, green	Lipid	75%	75%	75%	75%
1-Octen-3-ol	3391-86-4	Mushroom, earthy, broccoli	Broccoli, mushroom, earthy	Lipid	75%	0%	0%	100%
Alkyl Pyrazine 1 [H]	24168-70-5	Green, bell pepper, peapod	Green, vegetable, bell pepper, cilantro	Natural/ Protein [K]	100%	50%	75%	75%
Alkyl Pyrazine 2 [I]	24683-00-9	Green, peas, bell pepper	Bell pepper, broccoli, pea	Natural/ Protein	100%	100%	100%	100%
1-Octanol	111-87-5	Mushroom, green, vegetable	Grainy, vegetable, mushroom, musty	Lipid	50%	75%	75%	100%
1-Nonanol	143-08-8	Peas, vegetable, green,	Green, bell pepper	Lipid	100%	75%	75%	100%
γ-Caprolactone [J]	695-06-7	Candy, coconut, sweet	Sweet, coconut	Natural	50%	75%	75%	100%

[A] Percentage level based on the detection level identified by olfactory panelists. [B] Origin of VOCs from lipid oxidation, protein degradation or natural [8,10–12,17,39]. [C] N-W: Non-deflavored whole (unsieved). [D] Coarse/large, >250 µm (425 ≥ flour > 250). [E] Medium, >150 µm (250 ≥ flour > 150). [F] Fine/small, >106 µm (150 ≥ flour > 106). [H] Alkyl Pyrazine 1: 2-*sec*-butyl-3-methoxypyrazine. [I] Alkyl Pyrazine 2: 2-isobutyl-3-methoxypyrazine. [J] γ-Caprolactone: 5-ethyldihydro-2(3H)-furanone. [K] Natural/Protein: The origin of this compound is either natural or from protein degradation.

The intensity of VOCs varied based on the particle size and significantly ($p < 0.05$) impacted the TVI value (Table 1). The results of GC-O were consistent with the HS-SPME-GC results. Non-deflavored and deflavored coarse fractions exhibited a significantly ($p < 0.05$) lower TVI than the other samples. In contrast, the fine fraction had higher TVI values, although not significantly different from whole flour and the medium fraction (Table 1). However, the non-significant difference for TV values between coarse and medium fractions was observed in the HS-SPME-GC data (Table 1).

For all non-deflavored pea flours, both alkyl methoxypyrazines (Figure 2) were the most recognized compounds by the GC-O panelists owing to their low sensory threshold values, such as 3 ppt in the air for 2-*sec*-butyl-3-methoxypyrazine [44]. Methoxypyrazines have been reported as musty, green, and earthy off-aroma contributors of various plants (e.g., peas, asparagus, potatoes) with many descriptions (e.g., bell pepper, peapod, earthy, green) [8,44]. Nonanal was the only compound detected by GC-O for most deflavored flours, confirming the HS-SPME-GC analysis (Figure 1). Murat et al. [8] also reported this VOC in pea flour. The degree of intensity of other selected VOCs depended on the particle size. The low degree of intensity for all VOCs in the coarse fraction might be due to its higher bran content and less starch and protein components [20]. The strong volatile binding ability of proteins has been reported [8,33,39,41], thus, fractions with higher protein contents may have higher off-flavors. Among deflavored pea flours, the coarse fraction had zero degrees of intensity based on the GC-O analysis. Similarly, this fraction had the lowest TV based on the HS-SPME-GC analysis. Based on our previous report, the coarse fraction had the lowest protein and starch content compared to other fractions and whole pea flour [20]. The volatiles tend to bind to the protein and thus potentially less binding occurs in the lower protein fractions. Furthermore, the course fraction has less surface area to bind to compared to fractions with smaller particles.

3.3. QDA Analysis of Pea Flours

The sensory evaluation of pea flours using QDA was conducted to confirm the efficiency of SC-CO_2 extraction with different particle size pea flours on the removal of off-flavors (Table 1). Both factors (i.e., extraction and particle size) and their interactions showed significant effects ($p < 0.05$) on sensory attributes (i.e., pea intensity and bitterness) (Table 2). The range of pea intensity and bitterness in non-deflavored pea flours were between 63 to 112.3 mm and 26.7 to 65.5 mm, whereas those in deflavored pea flours were between 12 to 29.5 mm and 4.5 to 9.7 mm, respectively, based on a 147 mm line scale. These findings supported previous sensory data [10]. The significant reduction ($p < 0.05$) in pea intensity and bitterness of deflavored pea flours demonstrated that the SC-CO_2+EtOH

extraction effectively removed the selected VOCs, which agreed with HS-SPME-GC and GC-O findings (Table 1). Furthermore, Malcolmson et al. [4] determined the moderate pea aroma and slight bitter intensity in cooked yellow peas by QDA. Researchers determined various aroma descriptions (e.g., pea, metallic, grainy, earthy, vegetable, hay-like) for cooked peas [4].

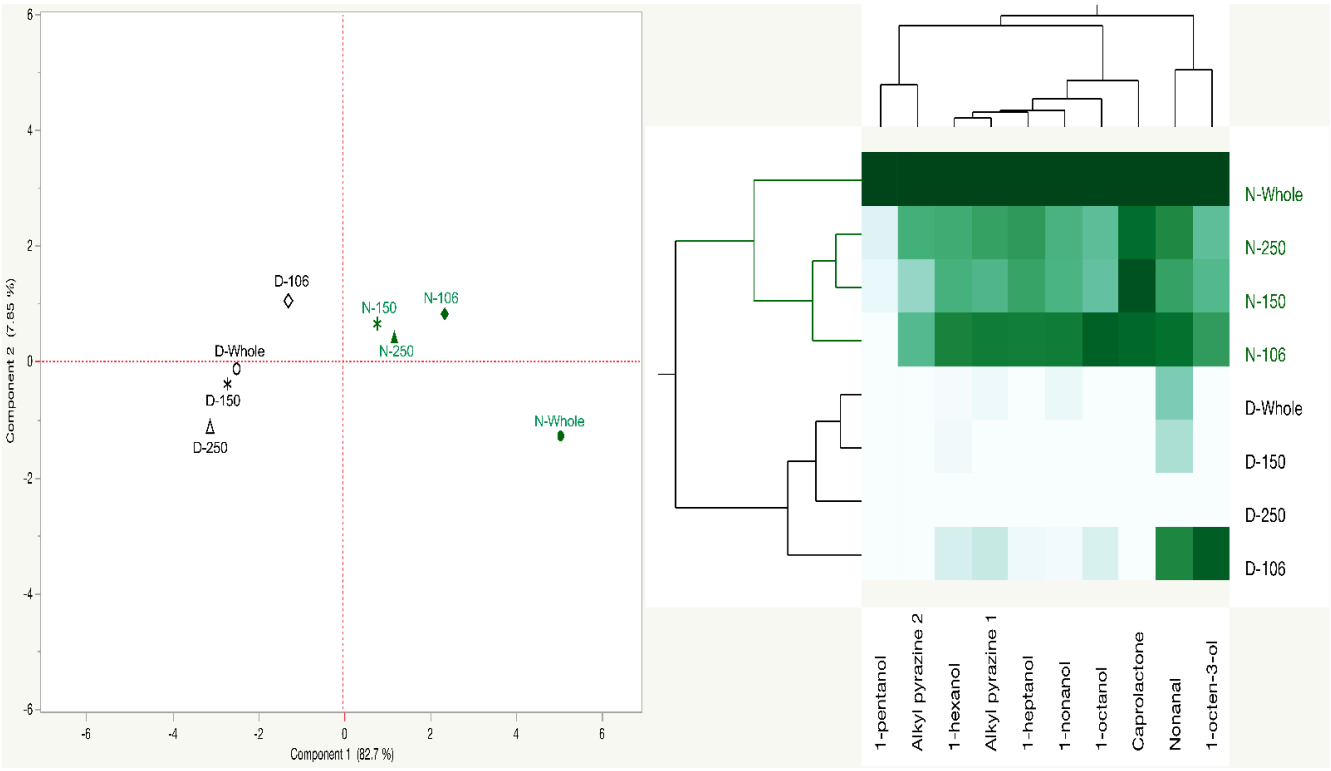
The undesirable bitter taste in dry peas is mostly associated with saponins, consisting of saponin B and saponin ßg (or called DDMP saponin) [10,11,18]. The SC-CO_2+EtOH extraction might promote the extraction of these saponins in the presence of high temperature and ethanol concentration, thereby resulting in decreased bitterness intensity for deflavored flours (Table 1). In addition, the likely conversion of DDMP saponin into less bitter saponin B might reduce the bitterness intensity (4.5 to 9.7 mm) in deflavored pea flours in the presence of ethanol and high temperature. A similar pattern was observed by Heng et al. [18]. In their study, the conversion of DDMP saponin, exhibited the higher bitterness intensity, into saponin B and maltol at >65 °C with ethanol [18]. The lower bitterness intensity (i.e., 4.5 to 9.7 mm) of deflavored pea flours indicated a possible conversion of DDMP saponins into less bitter saponin B and also their removal through this extraction with ethanol.

The particle size had significant impacts ($p < 0.05$) on pea and bitterness intensities. A relatively higher pea intensity and bitterness were recorded for the non-deflavored coarse fraction compared to medium and fine fractions (Table 1). These findings were negatively correlated with instrumental analyses. Potentially, panelists might chew coarse fractions for a longer time to reduce the particle size, resulting in an increased chance that off-flavor compounds might be perceived compared to other fractions. Thus, it may influence the perception and result in higher intensity. The coarse fraction might contain more hulls than other samples due to the increased resistance of the hull to size reduction [20]. Saponins in dry peas were reported at high concentrations in the hulls [39]. Therefore, the greater bitterness intensity in coarse fractions might be due to the higher hull content in this fraction. Furthermore, the metallic and astringent perception of saponins [11] may enhance pea intensity ratings for non-deflavored coarse fractions. Malcolmson et al. [4] reported a metallic aroma for cooked yellow peas.

Among samples, the non-deflavored fine fraction had the lowest pea intensity and bitterness. However, the instrumental analyses indicated relatively high TV and TVI amounts in the non-deflavored fine fraction (Table 1). This fraction was relatively higher in starch with a moderately high protein level [20]. Possibly, the greater flavor binding capacity of protein and starch might reduce the perception of off-aroma and might cause a longer time for the perception of VOCs during oral processing. The fine fraction had smaller particles; therefore, panelists may swallow this fraction relatively faster than the coarse fraction with less chewing and comminution. Subsequently, bolus formation, which is involved in jaw movement and saliva secretion, might be deficient and causes minimal interaction of particles with saliva and the oral cavity [45]. This can result in shorter retention times of the fine fraction in the mouth. In addition, fine and medium fractions that were higher in starch and protein contents [20] were relatively lower in hull particles, resulting in the lower bitterness intensity for these fractions compared to coarse and whole pea flours.

3.4. Chemometric Analysis of Response Variables from Instrumental and Sensory Analyses

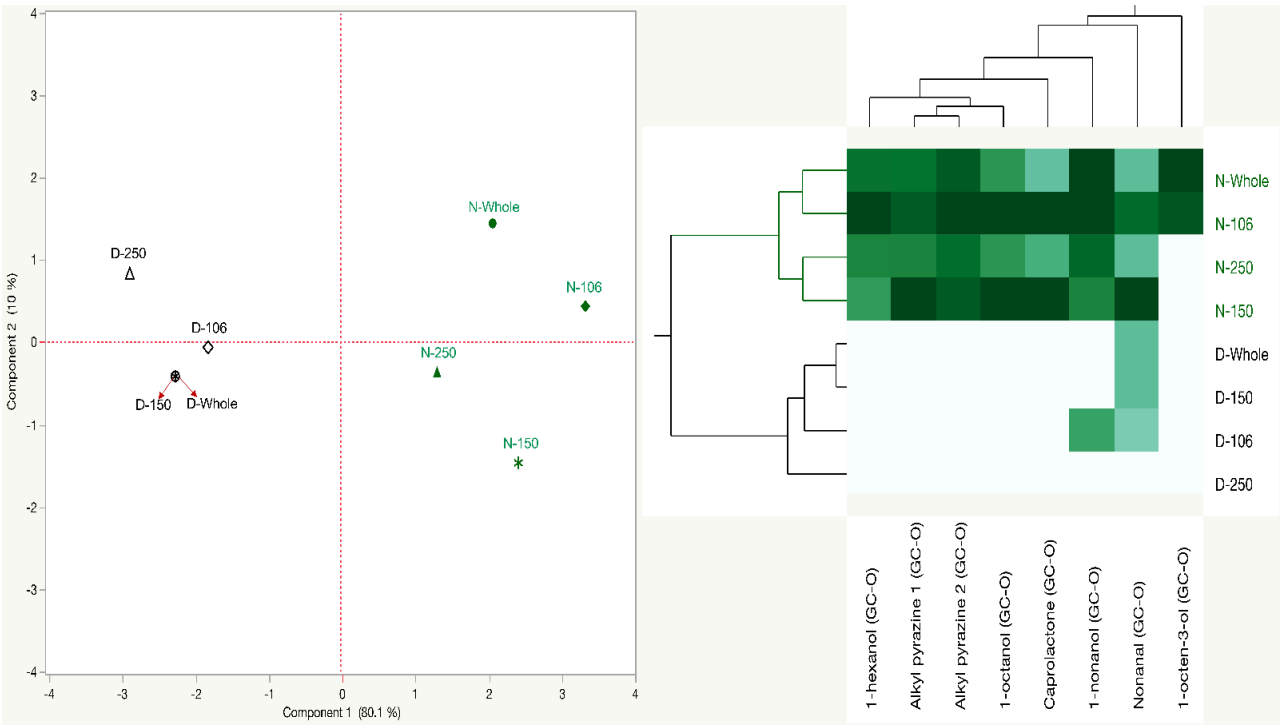
Among chemometric methods, PCA was employed on the correlation matrix to determine the complex interrelationships among response variables (i.e., flavor compounds and sensory attributes) via principal components (PCs) produced by reducing the dimensions of data with maximizing the variance. HCA was applied to identify the specific response variable (i.e., flavor compounds), accounting for the division of eight groups of pea flours in detail. The results of PCA and HCA were presented in Figures 3–5.



(**a**) (**b**)

Figure 3. Application of chemometric for volatile compounds detected by the HS-SPME-GC analysis. (**a**) Score plot of principal components 1 and 2 of the principal component analysis; (**b**) hierarchical cluster dendrograms of the hierarchical cluster analysis, where the color box presents the mean value of each response variable given on the *x*-axis. The white to dark green color represents a low to high level of response. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively, while D-counterparts are deflavored pea flours.

The PCA of VOCs from HS-SPME-GC (Figure 3a) illustrated that 82.7% of the total variance was explained by the first PC, while PC1 and PC2 explained 90.5% of the total variance. Based on this finding, PC1 explained all VOCs in one dimension, indicating a high correlation among VOCs. These outcomes agreed with a previous report [10]. Eight groups of pea flours were distributed on the positive and negative coordinates of PC1 based on responses of VOCs detected by HS-SPME-GC (Figure 3a). Non-deflavored medium and coarse fractions were strongly associated compared to the fine fraction and whole pea flour based on the VOCs profile (Figure 1). In particular, the non-deflavored whole pea flour was separate from other non-deflavored pea flours in the PCAs score plot. However, among deflavored flour samples that had a negative correlation with non-deflavored pea flours, the deflavored fine fraction was separated from other samples. This finding illustrated that decreasing the particle size reduced the efficiency of SC-CO_2+EtOH extraction. This observation can be related to the agglomeration of small particles, which reduces the diffusion of supercritical fluid during extraction. In addition, the negative correlation between deflavored and non-deflavored pea flour samples in the score plot of PCA supported the fact that this extraction was a promising deflavoring approach for pulse flours.



(**a**) (**b**)

Figure 4. Application of chemometric for volatile compounds determined by the GC-O analysis. (**a**) Score plot of principal components 1 and 2 of the principal component analysis; (**b**) hierarchical cluster dendrograms of the hierarchical cluster analysis, where the color box presents the mean value of each response variable given on the *x*-axis. The white to dark green color represents the low to high level of response. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively, while D-counterparts are deflavored pea flours.

Similarly, the VOCs determined by GC-O (Figure 4a) exhibited that PC1 was responsible for most variations (80.1%) across the samples, while PC2 explained only 10%, which explained a high correlation between the volatiles detected by GC-O. The VOCs of non-deflavored pea flours were positively correlated with the first PC, which was contrary to those of deflavored pea flours. Likewise, Chang et al. [32] found that the volatile profiles of LPI extracted with 95% of ethanol had a negative correlation with PC1. However, in this study, the VOCs of LPI extracted with lower ethanol concentration (35–75%) was positively correlated with PC1 due to containing higher off-aroma compounds. Similar findings were reported by Wang et al. [33] for deflavored PEPF samples extracted with higher ethanol concentration. Furthermore, a certain separation between non-deflavored and deflavored pea flours on the score plot of PCA (Figure 4a) was observed. This visual outcome indicated an effective SC-CO_2+EtOH extraction as a deflavoring process.

The cluster analyses of VOCs detected by HS-SPME-GC and GC-O showed that the non-deflavored and deflavored pea flours were broadly characterized into two groups based on the dendrograms of Figures 3b and 4b, respectively. The darkest green block represented the highest values of response variables, while the light (white-like) block was a non-value detected by the analysis. The SC-CO_2+EtOH extraction completely removed the selected off-aroma compounds from the coarse fraction and also diminished most of the VOCs from other pea flours (Figure 3b). Chang et al. [32] and Wang et al. [33] demonstrated a similar separation for the cluster analyses of LPI and PEPF after ethanol washing, respectively. Nonanal was the only VOC that was not entirely removed through the extraction and is represented by lighter green columns in deflavored flours and darker green columns in non-deflavored pea flours (Figures 3b and 4b). This aldehyde had a positive correlation with deflavored flours except for the deflavored coarse fraction (Figures 3b and 4b), which was related to a high affinity of proteins to aldehydes through

covalent bonds [41]. Similarly, nonanal was present at high amounts after ethanol washing of PEPF [33].

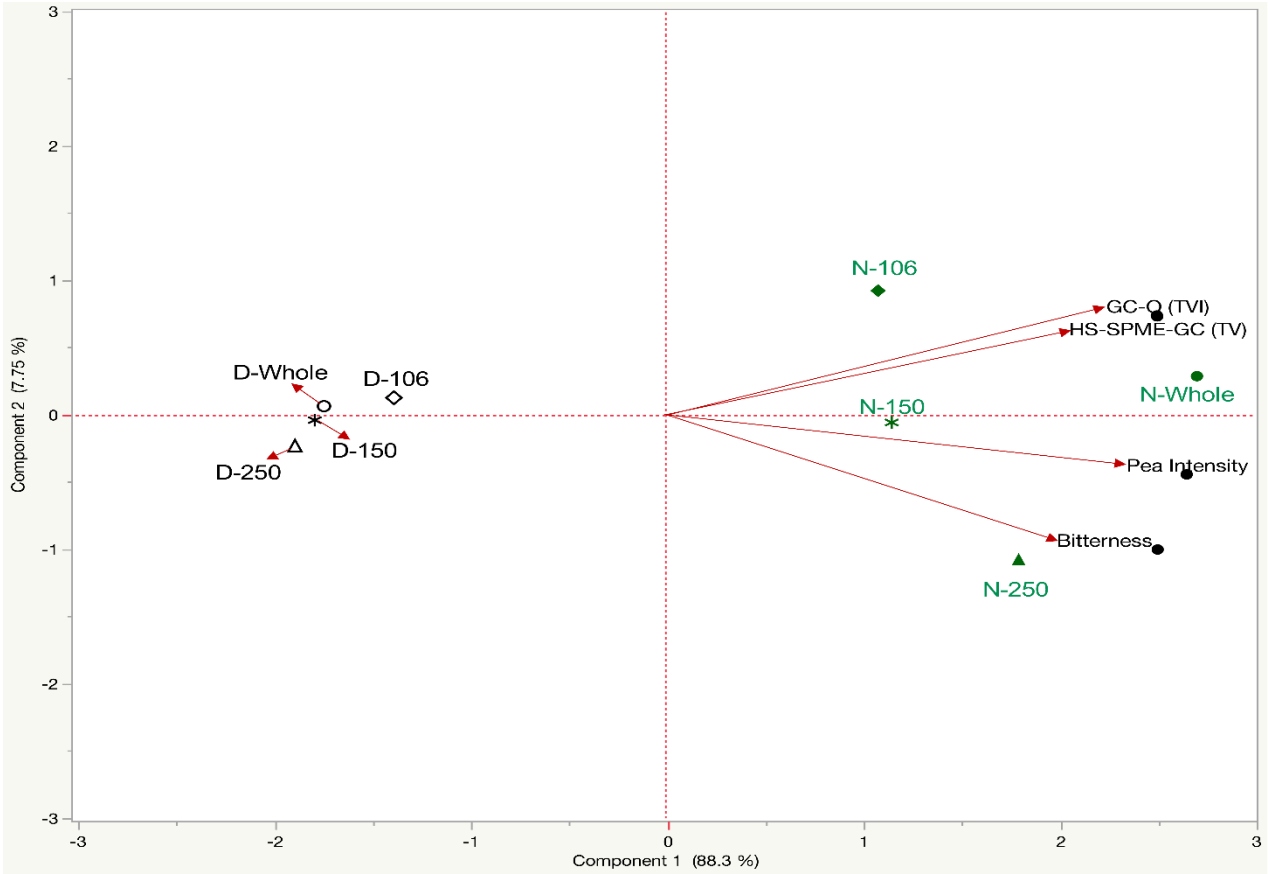


Figure 5. Principal component analysis of total volatile (TV) and total volatile intensity (TVI) detected by HS-SPME-GC and GC-O analyses, and sensory attributes using quantitative descriptive analysis (QDA), where the graph represents the biplot of principal components 1 and 2. N-Whole, N-250, N-150, and N-106 stand for non-deflavored whole (unsieved), coarse, medium, and fine pea flours, respectively, while D-counterparts are deflavored pea flours.

A combined PCA (Figure 5), including TV, TVI, and sensory attributes, was accounted for most variations (PC1, 88.3%) of the samples. This combined PCA biplot represented that all response variables were positively correlated with PC1, which had a negative correlation with deflavored pea flours. The pea intensity and bitterness had a close relation with non-deflavored whole flour and coarse fractions compared to non-deflavored medium and coarse fractions. However, deflavored pea flours had a negative correlation with all responses (Figure 5). The response variables located at the positive axes of PC1 along with non-deflavored pea flours represented the higher values recorded by the analyses for all PCA plots.

4. Conclusions

The impacts of SC-CO_2-EtOH extraction and particle size on the flavor profiles of pea flours were documented. Both factors had significant interaction effects for sensory attributes and instrumental outputs. The SC-CO_2-EtOH extraction significantly decreased off-aroma and off-taste compounds of all pea flour samples. Fractions with smaller particle sizes had higher off-aroma compounds, but fractions with larger particle sizes had higher bitterness intensity. The HS-SPME-GC and GC-O findings agreed with each other for non-deflavored and deflavored pea flours. However, the findings of instrumental analyses for the non-deflavored pea flour were opposite of the data from the sensory analysis. PCA revealed that volatiles were highly correlated with each other. The cluster analysis revealed

that non-deflavored and deflavored flours were separated based on the dendrograms. This study showed that flavor studies require multiple approaches to provide reliable results due to differences in the human flavor perception. The SC-CO_2+ETOH extraction could be an effective green technology to enhance the organoleptic properties of pulse ingredients.

Author Contributions: Conceptualization, S.V. and C.H.; methodology, S.V. and M.X.; validation, S.V., formal analysis, S.V.; investigation, S.V., M.X. and A.M.-L.; writing—original draft preparation, S.V.; visualization, S.V.; writing—review and editing, S.V., B.C. and C.H.; resources, B.C. and C.H.; supervision, C.H.; project administration, C.H.; funding acquisition, C.H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Office of Human Research Protections, in the Department of Health and Human Services (DHHS), in compliance with federal provisions (Protection of Human Subjects (45 CFR 46, 21 CFR 50)) and approved by the Institutional Review Board of North Dakota State University (Protocol AG18027, 9 November 2017 date of approval).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the Serap Vatansever or the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

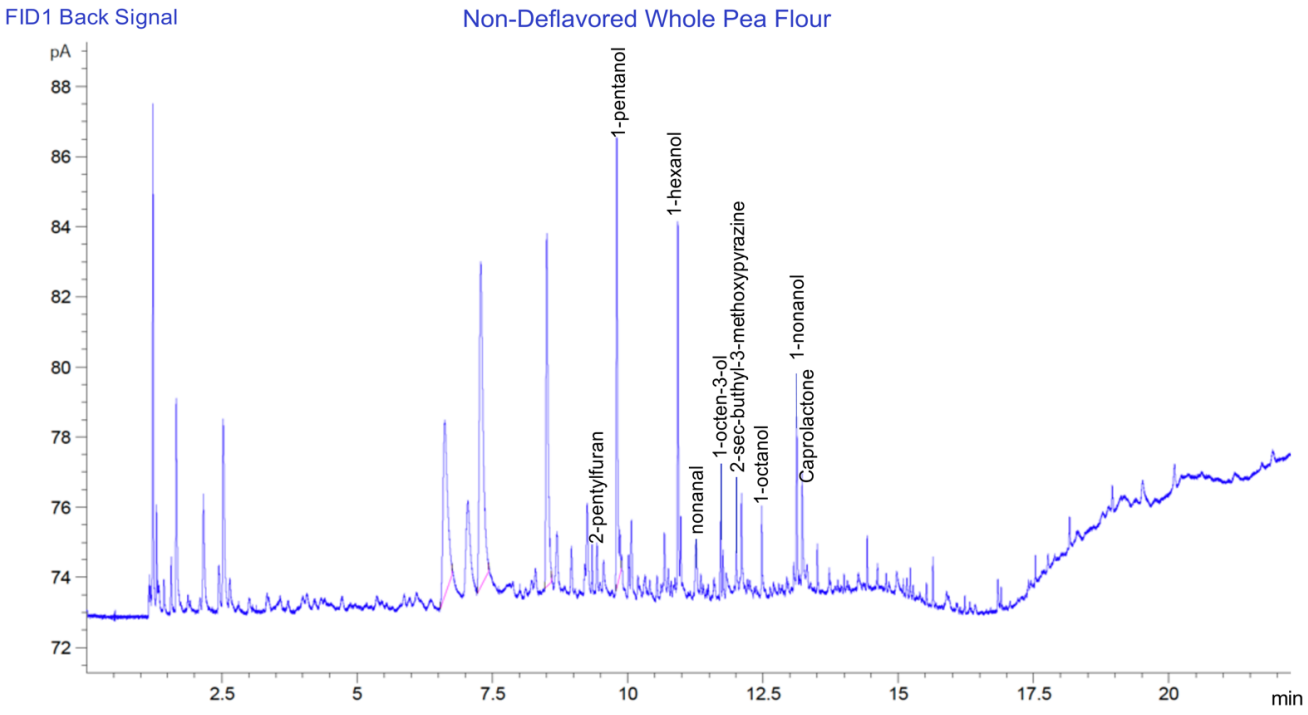

Figure A1. HS-SPME-GC/FID chromatogram of non-deflavored whole pea flour.

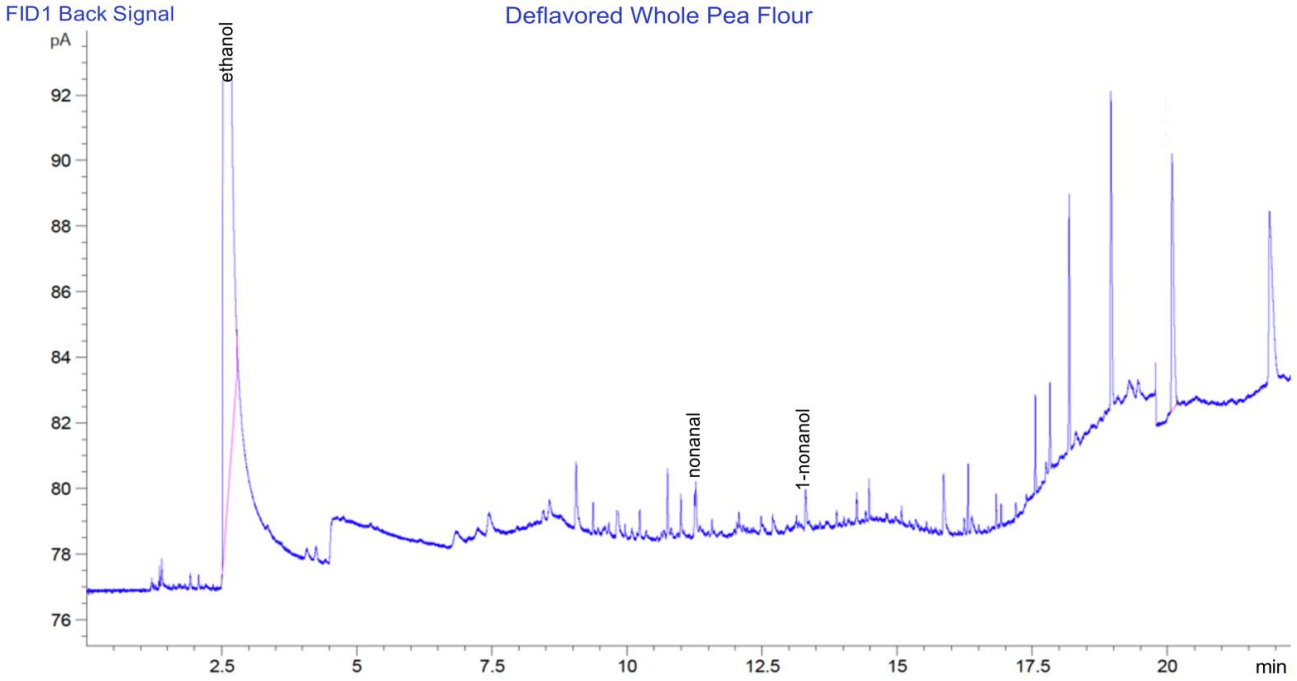

Figure A2. HS-SPME-GC/FID chromatogram of deflavored whole pea flour.

Table A1. Standard curve and threshold level of selected off-aroma compounds.

Off-Aroma Compounds	CAS Number	Threshold (μg/L) [A]	Standard Curve	R^2 [B]
2-pentylfuran	3777-69-3	6	y = 30.536x + 31.236	0.9746
1-pentanol	71-41-0	400	y = 2.8851x + 8.4212	0.9840
1-hexanol	928-96-1	2500	y = 9.0737x − 0.5495	0.9959
Nonanal	124-19-6	1	y = 7.3643x − 7.9437	0.9927
1-octen-3-ol	3391-86-4	1	y = 6.0098x + 0.6923	0.9869
1-heptanol	111-70-6	–	y = 5.338x − 0.6407	0.9919
2-*sec*-butyl-3 methoxypyrazine	24168-70-5	0.001	y = 2.028x − 0.1348	0.9990
2-isobutyl-3-methoxypyrazine	24683-00-9	0.001	y = 1.8039x − 1.5212	0.9846
1-octanol	111-87-5	110–130	y = 4.8329x + 1.1097	0.9933
1-nonanol	143-08-8	50	y = 1.2204x + 0.7846	0.9962
γ-caprolactone [C]	695-06-7	–	y = 4.1966x − 0.168	0.9976

[A] Odor threshold value in water. The data was retrieved from Leffingwell & Associates [46]. [B] R^2: Correlation coefficient of standard curve. [C] γ-Caprolactone: 5-ethyldihydro-2(3H)-furanone.

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