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Article

Monitoring the Aroma Profile during the Production of a Pea Protein Isolate by Salt Solubilization Coupled with Membrane Filtration

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ABSTRACT: The volatile profile was monitored during an optimized salt extraction process (salt solubilization coupled with membrane filtration) to produce a pea protein isolate (PPI). Aroma compounds from samples collected at different steps of the manufacturing process were isolated using solvent-assisted flavor evaporation (SAFE) and analyzed by gas chromatography−mass spectrometry−olfactometry (GC−MS−O) and GC−time-of-flight mass spectrometry (GC−TOF-MS). A sensory evaluation of pea flour (PF) and PPI aqueous solutions was also conducted. Twelve aroma compounds were perceived with a "moderate" odor intensity by panelists from the sniffing port of GC−MS−O. From the sensory evaluation, the aroma descriptors used to describe the PF and PPI testing solutions were also used to describe individual compounds eluting from the sniffing port. This observation supports the hypothesis that the 12 compounds identified in this study by GC−MS−O are likely to be the main contributors to the aroma profile of the samples analyzed.

KEYWORDS: *pea flour, volatile compounds, off-flavor, salt extraction, SAFE*

INTRODUCTION

In recent years, there has been an increase in demand for plant protein ingredients across the globe.[1] Among different plant protein sources, peas (*Pisum sativum* L.) have rapidly gained interest, mainly because peas are not genetically modified, they fix nitrogen in the soil, which reduces the need for fertilizers,[2] and they are suited to growing in much of North America. Additionally, peas have a low occurrence of allergenicity and are viewed as a healthier source of protein compared to meat and dairy products.[3,4] Despite the perceived health and environmental benefits, pea protein ingredients possess characteristic beany, grassy, and green notes, which have limited their utilization in food applications.[5] These off-notes are either generated by the plant itself (lipid, protein, and carbohydrate metabolism) or result from harvesting, processing conditions, and/or storage.[6]

Several reports on the intrinsic flavor of unblanched raw peas have been published as early as the late 1960s and 70s.[7−10] More recently, due to an increased demand for plant proteins as an alternative to animal proteins, there has been further interest in studying the aroma profile of blanched peas, pea flour (PF), pea protein isolate (PPI), and legumin and vicilin preparations (major pea protein fractions), and in understanding plant protein−aroma interactions.[11−13] Additional studies have focused on analyzing the impact of processing conditions on the aroma profile of pea ingredients and pea protein-based products. For instance, Trikusuma et al. characterized the changes in the aroma profile of a pea protein beverage submitted to ultra-high temperature (UHT) processing. They concluded that UHT processing significantly changed the volatile aroma composition and the sensory profile of the pea protein beverage. They mainly attributed these changes to two reaction pathways: lipid oxidation and the Maillard reaction.[14] Murat et al. identified the aroma compounds present at different steps during a pH-based extraction of pea protein by gas chromatography−mass spectrometry (GC−MS) but only analyzed the first and last step (PF and PPI) by GC−olfactometry (GC−O). Based on the GC−MS analysis and relative quantification, they indicated that the aroma profile evolved during the extraction process.[15] As one would expect, some compounds appeared, and others disappeared at different steps of the process.

Processing conditions have an important impact not only on the flavor quality but also on protein structural and functional properties. In order to make pea protein competitive in the market with other plant protein sources, such as soy protein, it is necessary to optimize the processing conditions to obtain PPIs with high protein purity (>80%), good functionality (solubility, emulsification, foaming, gelation, etc.), and free of off-flavor. To the best of our knowledge, there are no reports focused on monitoring the aroma profile during an optimized manufacturing process to produce PPIs. In preliminary work within our research group, the extraction conditions to maximize protein purity and yield following a salt extraction

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method were optimized.[16] Thus, the main objective of this study was to identify the volatile compounds present at each step of the optimized salt extraction process and monitor any changes in the aroma profile.

MATERIALS AND METHODS

Samples and Chemicals. The yellow field pea (*P. sativum* L.) flour was kindly supplied by AGT Foods (Regina, Canada). The flour was stored at room temperature in closed glass jars until use. Chemical standards of methyl hexanoate (99%), hexanal (98%), (*Z*)-4-heptenal (98%), (*Z*)-6-nonenal (95%), methional (98%), 1-octen-3-ol (95%), (*E*)-2-nonenal (97%), 3-methylbutanoic acid (isovaleric acid) (99%), hexanoic acid (99%), and maltol (98.5%) were purchased from Sigma-Aldrich (St. Louis, MO). 2-Isobutyl-3-methoxypyrazine (IBMP) (97%) and (*Z*)-2-octanol (98%) were obtained from AstaTech (Bristol, PA). (*E*)-2-Octenoic acid (98%) was purchased from TCI America (Portland, OR). A homologous series of straight-chain alkanes (C_5−C_{27}) and anhydrous magnesium sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM) (GC Resolv) (99.9%) was obtained from Fisher Scientific (Fair Lawn, NJ).

Extraction of PPI by Salt Extraction and Sample Collection. Figure 1 outlines the optimized protein extraction method that was

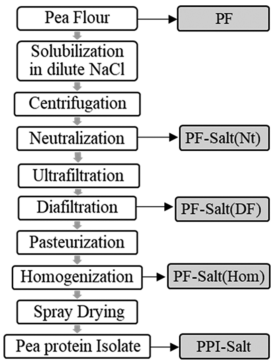

Figure 1. Samples collected along the extraction of pea protein following salt solubilization coupled with membrane filtration.

developed following salt solubilization coupled with membrane filtration.[16] The process starts by solubilizing the PF in dilute sodium chloride (0.5 M NaCl). This solution was agitated in a jacketed tank equipped with a stirrer (Vektor Series, Lightnin, Rochester, NY) for 1 h at room temperature (23 °C). The solution was passed through a decanting centrifuge (Westfalia Separator AG, CA 220-01-30, Oelde, Germany) and clarified using a desludging centrifuge (Westfalia Separator AG, SB 7-06-076, Oelde, Germany) in order to remove insoluble materials, such as starch and fibers. The separated liquid containing protein was set aside. The insoluble material was extracted again by adding dilute NaCl and agitated in a jacketed tank with an automated stirrer for 30 minutes. The solution was passed through the decanter centrifuge and desludging centrifuge. The combined liquid fractions were placed in a jacketed tank, the solution was neutralized with NaOH (6.25 M) and agitated until the pH was stable at pH 7.

The protein solution was then ultrafiltered (103−138 kPa inlet, 70−103 kPa outlet, PTI Advanced Filtration, PTI Technologies, St. Louis, MO) with tangential (cross) flow and a spiral wound membrane (3 kDa MWCO) and diafiltered to further concentrate the proteins by separating soluble fibers and small sugars. The retentate was pasteurized by passing the solution through a high temperature, short time (73 °C for 15 s) processing system (MicroThermics electric model 25HV Hybrid, 60−170 L/h, MicroThermics Inc, Raleigh, NC), followed by a two-stage homogenization (Gaulin 125 L; 17,200 kPa, 230 L/h, Manton-Gaulin Mfg. Co. Inc., Everett, MA). The homogenized retentate was then spray dried using a SPX Flow Anhydro spray dryer (9.5% TS, 180 °C inlet, 90 °C outlet, ca. 15 kg water evaporation per hour) with a wheel type atomizer (24,500 rpm) (SPX Flow Inc., Charlotte, NC).

Samples for flavor analysis were collected at different processing steps where we expected that the process may alter the aroma profile (Figure 1) {i.e., PF, after neutralization [PF-Salt(Nt)], after diafiltration [PF-Salt(DF)], after homogenization [PF-Salt(Hom)], and the final product or PPI (PPI-Salt)}. The samples were collected in glass jugs (3.8 L) and stored at −18 °C until further analysis.

Isolation of Volatile Aroma Compounds by Solvent-Assisted Flavor Evaporation. The amount of the sample used in flavor extraction at each processing step varied due to the dilution required by the process (some samples were very dilute while others were concentrated). Thus, the following sample amounts were used in extraction: 100.0 g for PF, 252.7 g for PF-Salt(Nt), 24.2 g for PF-Salt(DF), 21.8 g for PF-Salt(Hom), and 25.6 g for PPI-Salt. For the extraction of dry samples (i.e., PF and PPI-Salt), the sample was weighed into a 500 mL Erlenmeyer flask containing 250 mL of DCM and 100 μL of methyl hexanoate (0.2 mg/mL DCM) as an internal standard (ISTD). The suspension was stirred using a magnetic stirrer for 1 h at room temperature (23 °C). The suspension was then filtered to recover the DCM fraction (with extracted volatiles).

The method was modified slightly to work with a liquid sample. For liquid samples [PF-Salt(Nt), PF-Salt(DF), and PF-Salt(Hom)], DCM was added to the noted sample, the slurry was stirred for an hour and the DCM (with extracted volatiles) was collected and set aside. The extracted pea slurry was transferred back into the 500 mL Erlenmeyer flask, 250 mL of DCM was added again, and the solution was stirred for another hour at room temperature (23 °C). The solution was filtered (or decanted if clear), and the two solvent fractions were pooled. The pooled solvent extract obtained was introduced into a solvent-assisted flavor evaporation (SAFE) apparatus. SAFE extraction was carried out at 45 °C under vacuum (1.4×10^{-5} mbar). Once the SAFE extract was obtained, it was dried with anhydrous magnesium sulfate in order to remove water residues. Finally, the "dried" SAFE extract (without water residue) was concentrated to 50 μL by using a gentle stream of high-purity nitrogen.

GC−MS−O Analysis. An Agilent 6890N gas chromatograph-5973 MSD (mass selective detector) mass spectrometer equipped with a sniffing port was used for GC−MS−O analysis. The separation of volatile compounds was performed by using a fused silica capillary column DB-WAX (30 m length × 0.25 mm I.D × 0.25 μm film thickness, serial #UST510456H, Agilent Technologies, Inc). High-purity helium was used as a carrier gas at a constant flow of 3.5 mL/min. 2 μL of the sample was injected in the splitless mode. The oven temperature was programmed from 40 to 85 °C at a rate of 3 C°/min and from 85 to 220 °C at a rate of 5 °C/min with a final hold time of 3 min. The column effluent was split (1:1) using a fused silica T to feed the MS and the olfactometry port equal volumetric flows. The sniffing port transfer line from the splitting T (fused silica) was enclosed in a heated line maintained at 220 °C. The injection port and MS transfer line temperatures were 220 and 250 °C, respectively. The ionization energy was 70 eV, and the quality scan range was programmed to m/z 29−550.

Three panelists, who were all experienced with GC−O analysis, were recruited and instructed to record the retention time, the sensory descriptors of the volatile aroma compounds detected through the olfactometry port, and rate the odor intensity of each odorant using a general labeled magnitude scale (gLMS), where "no sensation" is at the left end and "strongest imaginable sensation" at the right end.[17]

The aroma compounds were tentatively identified using MS library (NIST—National Institute of Standards and Technology, version 2.2) matching and by the comparison of the calculated retention index (RI) with published values. The retention indices were calculated by spiking the sample extracts with a series of *n*-alkanes (C_5−C_{27}). Absolute identification was performed only for the aroma compounds that were rated with an average odor intensity ≥16.2 (corresponding to the descriptor "moderate") and detected by at least two panelists, as it was hypothesized that these compounds are likely

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Table 1. Volatile Aroma Compounds Extracted by SAFE and Identified at Different Processing Steps [PF: Pea Flour, PF-Salt(Nt): after Neutralization, PF-Salt(DF): after Diafiltration, PF-Salt(Hom): after Homogenization, and PPI-Salt: Pea Protein Isolate]

average RT (min)	aroma compound	calculated RI[a]	steps					identification[b]
			PF	PF-Salt (Nt)	PF-Salt (DF)	PF-Salt (Hom)	PPI-Salt	
			Aldehydes					
3.68	pentanal	977	+	+	+	+	+	R, MS
5.55	hexanal	1077	+	+	+	+	+	R, MS, S, O, TOF
6.64	(*E*)-2-pentenal	1119	+	+	+	+	+	R, MS, O
7.07	(*Z*)-3-hexenal	1135	+	+	+	+	−	R, MS, O
8.31	heptanal	1176	+	+	+	+	+	R, MS, O
9.29	(*E*)-2-hexenal	1207	+	+	+	+	+	R, MS
10.13	(*Z*)-4-heptenal	1231	+	+	+	+	+	R, MS, S, O, TOF
11.84	octanal	1277	+	+	+	+	+	R, MS, O
13.04	(*E*)-2-heptenal	1310	+	+	+	+	+	R, MS, O
15.76	nonanal	1384	+	+	+	+	+	R, MS, O
16.90	(*E*)-2-octenal	1416	+	+	+	+	+	R, MS, O
17.27	(*Z*)-6-nonenal	1427	+	+	−	−	+	R, MS, S, O, TOF
17.58	methional	1440	+	+	+	+	+	R, MS, S, O, TOF
18.86	(E,E)-2,4-heptadienal	1477	+	+	+	+	+	R, MS
19.65	benzaldehyde	1502	+	+	+	+	+	R, MS
20.19	(*E*)-2-nonenal	1520	+	+	+	+	+	R, MS, S, O, TOF
23.08	(*E*)-2-decenal	1631	−	+	+	+	−	R, MS
24.44	(E,E)-2,4-nonadienal	1683	+	+	+	+	+	R, MS, O
25.94	(E,E)-2,4-decadienal	1749	+	+	+	+	+	R, MS, O
27.16	tridecanal	1806	−	+	+	+	−	R, MS
31.50	pentadecanal	2030	−	−	−	+	−	R, MS
33.47	hexadecanal	2121	−	+	+	+	−	R, MS
40.50	vanillin	2538	+	+	+	+	+	R, MS
			Alcohols					
4.72	2-methylbut-3-en-2-ol	1033	+	+	+	+	+	R, MS
7.65	1-penten-3-ol	1153	+	+	+	+	+	R, MS
7.98	3-penten-2-ol	1165	+	+	+	+	+	R, MS
10.72	1-pentanol	1247	+	+	+	+	+	R, MS
13.22	(*Z*)-2-penten-1-ol	1316	−	+	+	+	+	R, MS
13.24	3-methyl-2-buten-1-ol	1316	+	+	−	−	+	R, MS
14.52	1-hexanol	1350	+	+	+	+	+	R, MS, O
15.60	(*Z*)-3-hexen-1-ol	1381	−	+	−	−	−	R, MS
17.05	2-octanol	1406	+	+	+	−	+	R, MS, S, O, TOF
17.94	1-octen-3-ol	1448	+	+	+	+	+	R, MS, S, O, TOF
18.12	1-heptanol	1453	+	+	+	+	+	R, MS
19.18	2-ethylhexan-1-ol	1486	+	+	+	+	+	R, MS, O
21.11	1-octanol	1554	+	+	+	+	+	R, MS
23.73	1-nonanol	1657	+	+	+	+	−	R, MS
28.32	1-undecanol	1856	+	−	−	−	−	R, MS
28.42	benzyl alcohol	1861	+	+	+	+	+	R, MS
29.11	phenethyl alcohol	1894	+	+	+	+	+	R, MS
30.39	1-dodecanol	1958	+	+	+	+	+	R, MS
36.09	1-pentadecanol	2268	−	+	+	+	+	R, MS
			Carboxylic Acids					
22.83	butanoic acid	1617	+	+	+	+	+	R, MS, O
23.82	isovaleric acid	1659	+	+	+	+	+	R, MS, S, O, TOF
26.05	methyl salicylate	1754	+	+	+	+	+	R, MS
25.44	pentanoic acid	1726	+	+	+	+	+	R, MS, O
27.81	hexanoic acid	1834	+	+	+	+	+	R, MS, S, O, TOF
30.05	heptanoic acid	1939	+	+	+	+	+	R, MS
32.12	octanoic acid	2046	+	+	+	+	+	R, MS
34.10	nonanoic acid	2153	+	+	+	+	+	R, MS
36.02	*n*-decanoic acid	2263	+	+	−	−	+	R, MS
			Ketones					
5.04	2,3-pentanedione	1049	+	+	+	+	+	R, MS, O
8.22	2-heptanone	1173	+	+	+	+	+	R, MS

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Table 1. continued

average RT (min)	aroma compound	calculated RI[a]	steps					identification[b]
			PF	PF-Salt (Nt)	PF-Salt (DF)	PF-Salt (Hom)	PPI-Salt	
			Ketones					
11.70	2-octanone	1274	+	+	+	−	+	R, MS
15.60	2-nonanone	1379	+	−	−	−	+	R, MS
16.17	3-octen-2-one	1395	−	+	+	+	+	R, MS
27.02	2-tridecanone	1799	−	−	+	+	−	R, MS, O
27.99	(*E*)-geranyl acetone	1843	+	−	−	+	+	R, MS
39.19	benzophenone	2454	+	+	+	+	+	R, MS
			Lactones					
24.26	gamma-caprolactone	1678	+	−	−	+	+	R, MS
31.31	gamma-nonalactone	2005	+	+	+	+	+	R, MS, O
			Terpenes					
8.69	D-limonene	1189	+	+	+	+	+	R, MS
			Furans					
9.87	2-pentylfuran	1224	+	+	+	+	+	R, MS, O
			Pyrazines					
20.08	2-isobutyl-3-methoxypyrazine or IBMP	1518	+	+	+	+	+	R, MS, S, O, TOF
			Esters					
25.51	ethyl undecanoate	1731	+	−	+	+	+	R, MS
			Sulfur Compounds					
29.85	benzothiazole	1930	+	+	+	+	+	R, MS, O
			Pyran					
30.13	maltol	1949	+	+	+	+	+	R, MS, S, O, TOF
			Others					
34.40	(*E*)-2-octenoic acid	2176	+	+	+	−	+	R, MS, S, O, TOF

[a]Retention indices. [b]Identification was done for each compound based on the following: R, the RI of the analyte matched the RI reported in the literature; MS, mass spectra of the analyte matched the NIST library spectra; S, mass spectra and the RI of the analyte matched those of an authentic standard; O, odor of the analyte matched the authentic standard and the description reported in the literature; TOF, GC-TOF-MS was used to identify the compound and match its identity to the NIST library. "+" Compounds detected by GC−MS in the sample; and "−" compounds not detected by GC−MS in the sample.

to be the most significant contributors to the aroma profile of the samples. Absolute identification was conducted by comparing mass spectra, the RI of the compounds in the sample with those of the pure aroma standards, and odor descriptors with their corresponding standards and the literature.

Relative quantification was carried out by integrating the area under the curve (AUC) for each identified aroma compound. The area of each aroma compound was then normalized using the average area of the ISTD across all samples. Each aroma isolate was run in triplicate in GC−MS−O.

GC−Time-of-Flight MS Analysis. In order to confirm the identity of the aroma compounds that had an average odor intensity ≥16.2 and that were found through GC−MS−O, a GC−time-of-flight MS (GC−TOF-MS) analysis was carried out. The SAFE extracts obtained from each sample [PF, PF-Salt(Nt), PF-Salt(DF), PF-Salt(Hom), and PPI-Salt] were combined and concentrated to 50 μL by using a gentle stream of high-purity nitrogen. An Agilent 7890A Gas Chromatographic system (Agilent Technologies, Santa Clara, CA) coupled to Pegasus 4D TOF-MS (LECO Corporation, St. Joseph, MI) was used. The separation of volatile compounds was performed using a fused silica capillary column DB-WAX (30 m length × 0.25 mm I.D × 0.25 μm film thickness, serial #US0570343H, Agilent Technologies, Inc). High-purity hydrogen was used as a carrier gas at a constant flow of 3 mL/min. 1 μL of the sample was injected in the splitless mode. The oven temperature was programmed from 40 to 85 °C at a rate of 3 C°/min and from 85 to 220 °C at a rate of 5 °C/min with a final hold time of 3 min. The injection port and transfer line temperatures were 220 and 250 °C, respectively. The ionization energy was 70 eV, and the quality scan range was programmed to m/z 29−400 at a scan rate of 20 scan/s. Data processing was carried out using ChromaTOF software (version 3.4).

The compounds were tentatively identified by comparison with mass spectrometric data from the NIST library version 2.2.

Sensory Evaluation. This sensory evaluation was conducted in compliance with the University of Minnesota Institutional Review Board (STUDY00011991). The samples used for the sensory evaluation were as follows: 10% PF aqueous solution and 10% PPI (PPI-Salt) aqueous solution. Thirty milliliters of each aqueous solution were placed in a clear 120 mL sample cup with lid and were served at room temperature (28 °C). The samples were assigned three-digit codes. Eight panelists (37% men and 63% women) from the Department of Food Science and Nutrition at the University of Minnesota served as judges, all of whom have experience in sensory analysis. The sensory evaluation of the samples occurred over one session lasting 1 h. Participants were provided the two solutions and were instructed to smell each sample and record the odor descriptors and each odor descriptor's intensity. The intensity of each attribute was rated by using a gLMS where 0 corresponds to "no sensation" (at the left end of the scale) and 100 corresponds to "strongest imaginable sensation" (at the right end of the scale).[17]

Statistical Analysis. The analysis of variance was performed using R Studio software version 1.4.1103 (R Studio, Inc., Boston, MA). Significant differences ($p \leq 0.05$) between the mean ($n = 3$) values of the three injections of the same aroma isolate in GC−MS−O were determined by using a Tukey−Kramer honestly significant difference multiple means comparison test.

RESULTS AND DISCUSSION

Volatile Aroma Compounds Identified by GC−MS−O during Pea Protein Extraction. The aroma compounds extracted from samples and identified by GC−MS−O analysis are shown in Table 1. In total, 60 volatile aroma compounds

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were identified in PF-Salt(Nt), 58 in PF-Salt(DF), PF-Salt(Hom), and PPI-Salt, and 57 in PF. The aroma compounds extracted from the samples belonged to several different chemical classes, with aldehydes being the most abundant, followed by alcohols, carboxylic acids, and ketones. These four chemical groups are often found in peas and have been previously reported in other studies.[8,11,12] Other chemical species including lactones, terpenes, furans, pyrazines, esters, sulfur compounds, and pyrans were identified in the samples in smaller proportions.

In this study, the focus was put on the aroma compounds that were rated with an average odor intensity ≥16.2 (moderate) and detected by at least two panelists in at least one of the steps of the process, as these compounds are likely to be the most significant contributors to the aroma profile of the samples. The 12 volatile compounds that met these requirements are presented in Table 2. The quantitative data on these compounds at various steps in processing are also shown in Figure 2.

Table 2. Aroma Compounds Rated with an Average Odor Intensity ≥16.2 by at Least Two Panelists in at Least One of the Steps of the Pea Protein Extraction Process

#	aroma compound	description by panelists	description found in the literature
1	hexanal	green, grassy	green, grassy, leafy[11,15]
2	(*Z*)-4-heptenal	oily, fatty, fishy, oxidized oil	oily, fatty, cream-like, fishy[28,29]
3	2-octanol	grassy, musty, moldy, earthy	green, woody, herbal, earthy[28]
4	(*Z*)-6-nonenal	raw cucumber, celery, beany	green, cucumber, vegetable[28]
5	methional	raw potato, vegetable	potato, vegetable, musty[14,15,28]
6	1-octen-3-ol	mushroom, brothy	mushroom, fungal, musty[15,30]
7	2-isobutyl-3-methoxypyrazine or IBMP	bell pepper, earthy, soil	green bell pepper, pea[8,28]
8	(*E*)-2-nonenal	cucumber, nutty	fatty, cucumber[28,31]
9	isovaleric acid	cheesy, sour, pungent	cheesy, sweaty[14,15]
10	hexanoic acid	cheesy, pungent, rancid	fatty, cheesy[15,28,31]
11	maltol	sweet, caramel	sweet, caramellic[14,28]
12	(*E*)-2-octenoic acid	musty, moldy, dirty	musty, fatty, dirty, cheesy[28]

From PF to the following step, neutralization [PF-Salt(Nt)], the concentrations of most of the aroma compounds increased [except for methional and (*Z*)-6-nonenal]. These apparent increases in the concentration are likely due to the PF being a solid material (structurally intact), which would limit the extraction efficiency of the volatile compounds as compared to later samples in which the pea plant structure was broken down by the extraction process. Data interpretation on volatile concentrations at subsequent processing steps may be complicated by changes in sample pH, protein denaturation, and/or the presence of salt in the solution. There is substantial information in the literature on how the binding of aroma compounds by plant proteins is influenced by the noted factors.[18] Thus, some of the variation in the measured volatile concentration may reflect issues in volatile extraction from the protein (solution) rather than the absolute amount of aroma compounds in the sample. We are not aware of any research which has investigated the ability of SAFE extraction to recover aroma compounds when bound to proteins. Because there is no way to correct for this potential analytical complication, we will continue the discussion of the data as obtained.

After neutralization, a two-step filtration [the PF-Salt(DF) sample] was performed in order to concentrate the protein and remove low-molecular weight components (salts and sugars). Along with these low-molecular weight components, some of the aroma compounds appear to have been lost, except for (*Z*)-4-heptenal which remained constant. When sample dilution is done, compounds with significant water solubility would also be diminished.

Following the two-step filtration, the protein solution was subjected to pasteurization (for food safety reasons) and homogenization. These two processes were carried out in a closed system where one might think there was no loss of volatile compounds. However, the level of some of the aroma compounds, including hexanal, 2-IBMP, isovaleric acid, and hexanoic acid, decreased, as indicated in the PF-Salt(Hom) sample. The levels of methional increased, and the concentrations of (*Z*)-4-heptenal, 1-octen-3-ol, (*E*)-2-nonenal, and maltol remained unchanged. At this stage of the process, protein is the main component that is left in the solution as most of the other components have already been removed. The last step of the process was spray drying. During this process, a substantial decrease in the levels of some of the aroma compounds was observed. Losses may be attributed to evaporation during spray drying or enhanced binding with the proteins due to exposure to high temperatures reducing volatile recovery.[18,19]

Overall, when comparing PF (beginning material) with PPI-Salt (final PPI), it is observed that the levels of most of the aroma compounds significantly decreased. This observation is in agreement with previous reports on soy proteins.[20] The authors of this early study suggested that soy protein concentrates have a reduced flavor level compared to soy flour due to the removal of flavor compounds during the concentration processes.

Odor Description and Intensity of Aroma Compounds Identified During the Production of Pea Protein by Salt Extraction. Each of the samples collected at different steps along the manufacturing process of protein isolates were analyzed through the sniffing port coupled to the GC−MS system. As mentioned previously, the compounds shown in Table 2 are the main focus of this research due to their odor intensity of ≥16.2.

Isovaleric acid, hexanoic acid, and 2-octanol were detected with an average odor intensity ranging between 16.2 and 33.1 (which corresponds to "moderate" and "strong", respectively, on the gLMS) by the panelists as shown in Figure 3. The two carboxylic acids were detected by the panelists in all the samples and were described by the panelists as having "cheesy, sour, pungent, and rancid" notes. In a previous study, isovaleric acid was found in the PF and was described by panelists as having "animal" note. In the same study, hexanoic acid was also found, but its odor was described as having "feces, meat broth, and sewer" notes.[15] 2-Octanol was similarly detected by the panelists in all the samples and was described as having "grassy, musty, moldy, and earthy" notes. This compound has been previously found by other researchers in frozen green peas.[8]

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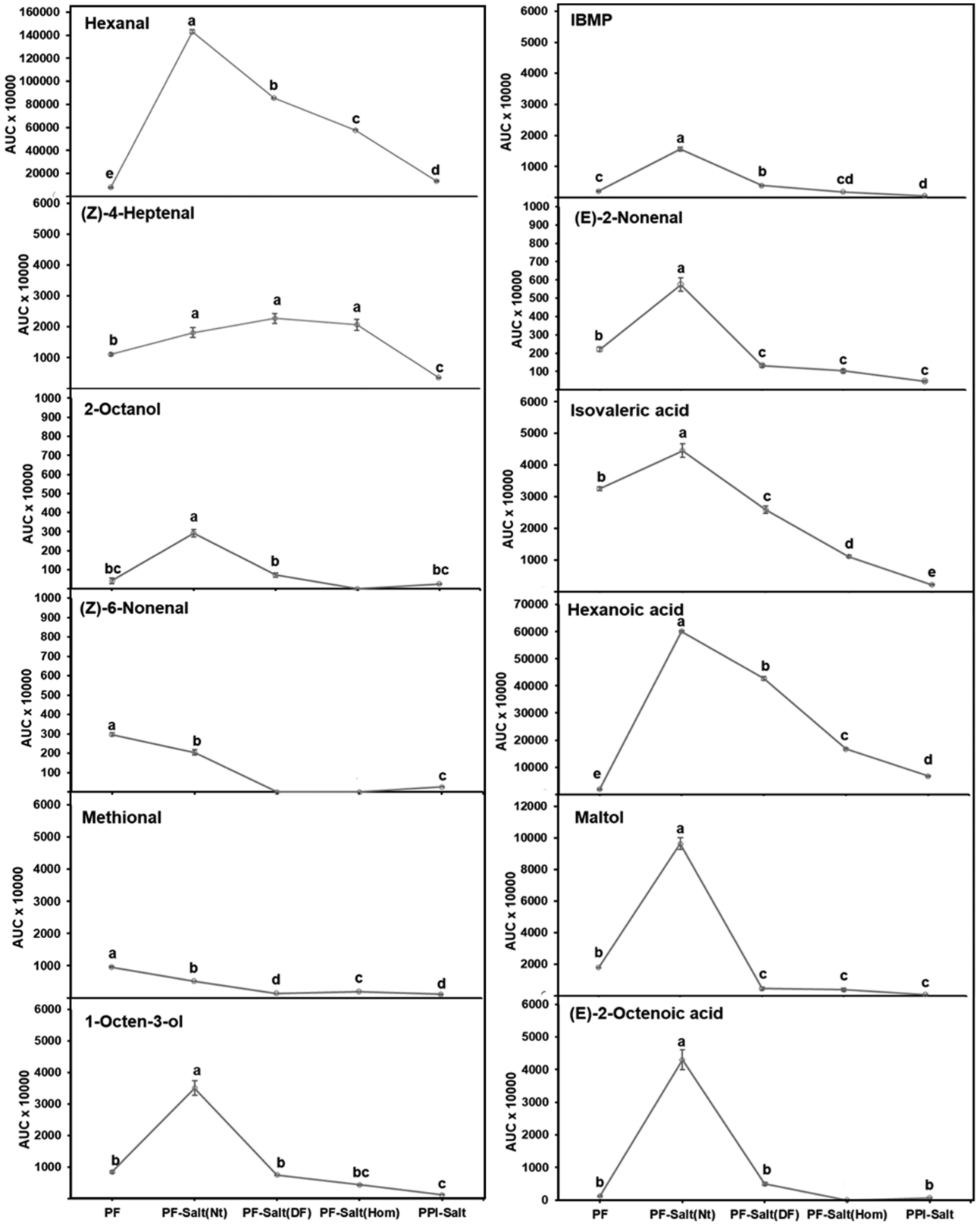

Figure 2. AUC of aroma compounds present in samples collected at different steps of the pea protein extraction process. The error bars represent the standard error of the mean (n = 3) values of three injections of the same aroma isolate in GC−MS−O. Different lowercase letters above the bars indicate significant differences of each aroma compound across processing steps and according to the Tukey−Kramer multiple means comparison test (P < 0.05).

Methional and IBMP were rated with an average odor intensity close to 16.2 (which corresponds to "moderate" on the gLMS). Methional was detected by the panelists in all the samples except in PF-Salt(Hom) and was characterized as

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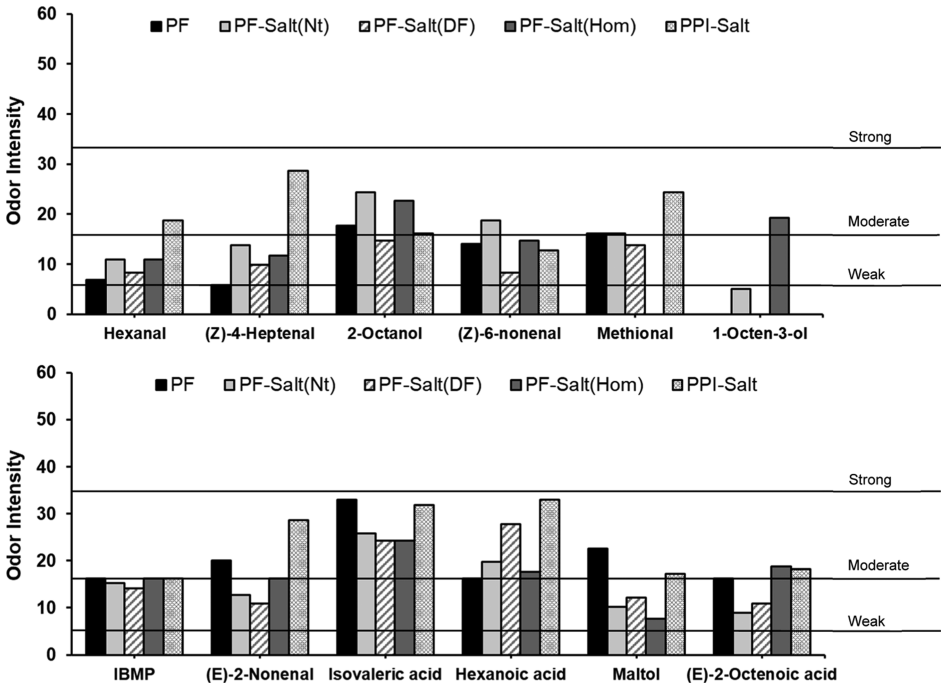

Figure 3. Mean of the perceived odor intensity for the 12 most significant aroma contributors present in samples collected at different steps during the pea protein extraction process. Intensity ratings are from the 100 point gLMS; a rating of 5.8 corresponded to the descriptor "weak", a rating of 16.2 corresponded to the descriptor "moderate", and a rating of 33.1 corresponded to the descriptor "strong".

having "raw potato" notes. In previous studies, methional was detected by panelists in pea protein extracts[15] and pea protein beverages.[14] In both studies, this compound was described by the panelists as having "potato" and "boiled potato" notes. IBMP was detected by panelists in all the samples and was described as having "bell pepper, earthy, and soil" notes. This compound has previously been found in frozen green peas, blanched green peas, PF, and pea protein beverages.[8,11,14,21]

Aroma compounds including hexanal, 1-octen-3-ol, (*E*)-2-nonenal, and maltol were rated with an average odor intensity of between 5.8 and 16.2 (which corresponds to "weak" and "moderate", respectively, on the gLMS scale). Hexanal was detected by the panelists in all the samples and was characterized as having "green and grassy" notes. Hexanal has been the most common compound found in raw peas and pea ingredients by previous researchers. Hexanal is often described as having "fresh and grassy" notes.[8,11,12,14,15,22,23] 1-Octen-3-ol was detected only in two samples [PF-Salt(Nt) and PF-Salt(Hom)] and was characterized as having"mushroom and brothy" notes. 1-Octen-3-ol was detected in the PF and PPI by panelists in a previous report, and was characterized as having "mushroom and vegetable" notes.[15] (*E*)-2-Nonenal was detected by panelists in all the samples. This compound was characterized as having "cucumber and nutty" notes. The presence of (*E*)-2-nonenal in frozen green peas and the PF has previously been reported.[8,12] Maltol was detected by panelists in all the samples. Panelists characterized this compound as having "sweet and caramel" notes. The presence of maltol in pea protein beverages has been previously reported.[14]

Other aroma compounds including (*Z*)-6-nonenal, (*E*)-2-octenoic acid, and (*Z*)-4-heptenal are reported for the first time in this study. These compounds were detected by all panelists in all of the samples. (*Z*)-6-Nonenal was described as having "raw cucumber, celery, and beany" notes, (*E*)-2-octenoic acid as having "musty, moldy, and dirty" notes, and (*Z*)-4-heptenal as having "oily, fatty, fishy, and oxidized oil" notes.

Sensory Evaluation. A sensory evaluation of aqueous solutions of the starting material (PF) and the final product (PPI-Salt) was conducted in order to look for relationships between the GC−MS−O data and overall perception. In the sensory evaluation, the aroma descriptors listed by at least two panelists are shown in the bar graph presented in Figure 4.

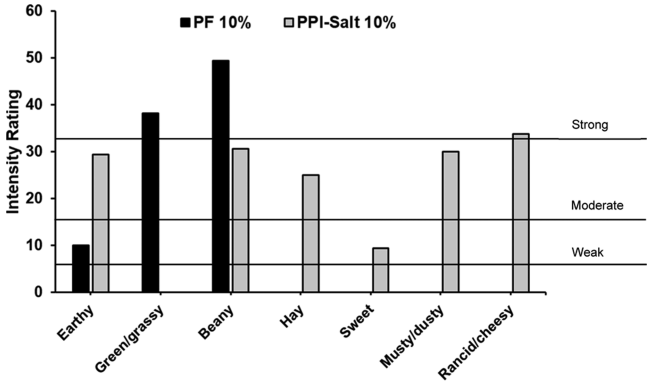

Figure 4. Mean intensity ratings of PF and PPI-Salt aqueous solutions tested for aroma. Intensity ratings are from the 100 point gLMS; a rating of 5.8 corresponded to the descriptor "weak", a rating of 16.2 corresponded to the descriptor "moderate", and a rating of 33.1 corresponded to the descriptor "strong".

Predicting a final overall sensory character of a food based on individual odors eluting on GC−MS−O analysis is highly unlikely. However, it is encouraging to see that the majority of aroma descriptors used in sensory analysis were also used to describe individual aroma compounds eluting from the sniffing port—some individual sensory notes could be linked to individual aroma compounds (Table 2). For example, the

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compounds responsible for the "earthy" notes in the tasting solutions were likely 2-octanol and IBMP. The "green/grassy" aroma was likely from the presence of hexanal and 2-octanol. The "beany" odor character was due to (*Z*)-6-nonenal and the "sweet" note was from maltol. The "musty/dusty" aroma could be attributed to 2-octanol and (*E*)-2-octenoic acid. The "rancid/cheesy" note was likely due to isovaleric and hexanoic acid.

Figure 4 shows that the hay, sweet, musty/dusty, and rancid/cheesy notes were used to characterize PPI-Salt but not the PF. Two possible explanations for these results are as follows: (1) the free amounts of the compounds responsible for these aromas were present at low concentrations in the PF but increased during the salt extraction of the protein to the point that panelists were able to detect them in PPI-Salt. However, this cannot be correlated with the analytical data of this study because DCM was used for the extraction of the compounds rather than water. (2) Suppression effect: the relatively high intensity of green/grassy and beany notes in the PF could have caused a suppression effect on the other aromas (hay, sweet, musty/dusty, and rancid/cheesy).

Theoretical Pathways of Aroma Compound Formation. As shown in Table 3, most of the volatile compounds are known to originate from either the enzymatic or autoxidative degradation of lipids. Lipoxygenases are enzymes that occur naturally in peas.[15] These enzymes catalyze the oxidation of fatty acids which, after undergoing a series of reactions, result in the formation of secondary products including aldehydes, ketones, furans, and alcohols.[24] The lipid content of field peas ranges between 1.2 and 6.3%. Linoleic acid (C18:2) is the major fatty acid in pea oil (46%), followed by oleic (C18:1) and linolenic acid (C18:3) with 31 and 11%, respectively.[25] Despite the low lipid content in peas, the degradation of these fatty acids during the manufacturing of PPIs is likely responsible for the formation of most of the aroma compounds found in PPIs.

Table 3. Formation Pathway of Volatile Aroma Compounds Detected during Manufacturing of the PPI

aroma compound	formation pathway	sources
hexanal	lipid oxidation	32
(*E*)-2-nonenal	lipid oxidation	32
1-octen-3-ol	lipid oxidation	8, 33
hexanoic acid	lipid oxidation	34
(*Z*)-6-nonenal	lipid oxidation	35
2-octanol	lipid oxidation	36, 37
(*Z*)-4-heptenal	lipid oxidation	38, 39
(*E*)-2-octenoic acid	lipid oxidation	40, 41
methional	Strecker degradation	42
2-isobutyl-3-hydroxypyrazine (IBMP)	Maillard reaction	10, 14
maltol	Maillard reaction	43, 44
isovaleric acid	amino acid metabolism	45, 46

A few other compounds including methional, IBMP, and maltol have been reported to be products of the Maillard reaction and associated Strecker degradation. These compounds were detected in the samples before any thermal treatment was applied, which would seem unusual. However, the Maillard reaction does take place at low temperatures, but at a much slower rate.[26,27]

In conclusion, processing treatments used during an optimized salt extraction of the pea protein led to variations in the levels of the most significant contributors to the aroma profile of the samples examined. The variations in the levels of some of the aroma compounds at various stages of the isolation process appeared to be noted by the panelists sniffing the GC−MS effluent. Additionally, the majority of the descriptors used in the sensory evaluation were also used during the olfactory analysis. This finding supports our hypothesis that the 12 aroma compounds identified through instrumental analysis likely contribute to the aroma profile of the samples. None of the major odorants were newly formed or completely lost during the protein isolation process, suggesting that the processing steps do not completely remove existing or generate significant new aroma compounds. These observations suggest that the aroma compounds identified in the samples may come from the normal metabolism of the peas, be produced during the storage of the peas, and/or be produced during the process of obtaining the PF.

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Notes

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ABBREVIATIONS

UHT, ultra-high temperature; GC−MS−O, gas chromatography−mass spectrometry−olfactometry; GC−TOF-MS, gas chromatography−time-of-flight mass spectrometry; ISTD, internal standard; NaCl, sodium chloride; PF, pea flour; Nt, neutralization; DF, diafiltration; Hom, homogenization; PPI, pea protein isolate; SAFE, solvent-assisted flavor evaporation;

RI, retention index; gLMS, general labeled magnitude scale; IBMP, 2-isobutyl-3-methoxypyrazine

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