Volatile profiles of commercial vetch prepared via different processing methods

Samuel Riley [a,b], Aneesh Lale [d,f], Vy Nguyen [b], Hangwei Xi [b], Kerry Wilkinson [c], Iain R. Searle [b], Ian Fisk [a,e,*]

[a] *Division of Food Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington LE12 5RD, UK*
[b] *School of Biological Sciences, The University of Adelaide, Adelaide, South Australia 5005 Australia*
[c] *School of Agriculture, Food and Wine and Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, South Australia 5064, Australia*
[d] *School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK*
[e] *The University of Adelaide, North Terrace, Adelaide, South Australia, Australia*
[f] *Future Food Beacon of Excellence, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK*

ARTICLE INFO

ABSTRACT

Vicia sativa (Common Vetch) is currently an underutilised leguminous crop species with high protein content and superior drought tolerance. This study aimed to understand the mechanisms behind vetch flavor development following processing to facilitate its uptake as a future source of dietary protein. A total of 95 volatile compounds were identified by solid-phase microextraction gas chromatography-mass spectrometry (SPME GC–MS) for a range of vetches processed by dehulling, soaking, germination, microwaving, and fermentation. 2-pentyl furan, benzyl alcohol, benzaldehyde, 1-octen-3-ol and 1-hexanol were found to be characteristic aroma compounds of *V. sativa*. Analysis of a *V. sativa* landrace demonstrated significant intraspecies variation in volatile abundance, three-fold that of commercial varieties. Both natto and tempeh fermentation produced significant quantities of alcohols, esters, and carboxylic acids with specifically natto generating significant pyrazines. Concentrations of 1-octen-3-ol significantly decreased after tempeh fermentation indicating its potential to reduce documented off flavor generating volatiles within *V. sativa*.

1. Introduction

The global population is growing at an exponential rate, expected to exceed nine billion people by 2050 (Roberts, 2011). As a result, demand for economical, sustainable and high-quality protein is expected to increase. Global protein currently relies on conventional animal sources; however, the production process is inherently space inefficient, water intensive and wasteful as large proportions of the biomass produced is indigestible. Instead increased global consumption of plant-based proteins, primarily that of pulses such as lentil (*Lens culinaris*), faba bean (*Vicia faba*), and soybean (*Glycine max*) has been prioritized by the highly significant EAT-Lancet Commission summary report (The Eat-Lancet Commission, 2019). Leguminous crops have inherent agricultural benefits such as being able to improve soil fertility by fixing atmospheric nitrogen, hence reducing nitrogen fertilizer input. In addition, the seed of legume crops are highly nutritious and have been historically used as a rich protein food source for both human and

animals. However, global pulse consumption only accounts for 7.5% of total daily protein in developing countries and only 2.5% in developed countries with their agricultural production and consumption dwarfed by that of carbohydrate cereal crops (Eicher, 2006). As the consideration of legumes as sustainable protein sources increases, research needs to be expanded beyond conventional pulses and focus upon the usage of previously underutilized species. The species within the *Vicia* genus have rapidly become some of the most internationally prominent legume crops, particularly *Vicia sativa* (common vetch) which is known for its excellent drought tolerance, (Enopala et al., 2012), high crude protein content (24–32%) (Valentine & Bartsch, 1996) and relatively low proportions of primarily unsaturated lipids (1.5–2.7%) (Mao et al., 2015). Historically, *V. sativa* was widely disregarded as a human food crop due to the presence of anti-nutritional factors in the seed; instead, it was used within crop rotation and was only suitable for ruminant feed. Currently, two anti-nutritional factors, principally the dipeptide γ-glutamyl-β-cyanoalanine (GBCA) and to a lesser extent, the free amino acid

β-cyano-L-alanine (BCA), exist at concentrations within the raw untreated seed that are associated with neurotoxicity in monogastric species (Valentine & Bartsch, 1996). As understanding of the toxin accumulation within *V. sativa* improves, the establishment of a zero-toxin vetch line is now being actively pursued and as a result, *V. sativa* is likely to be a significant contributor to future global protein demand (Nguyen et al., 2020).

To develop an edible high protein *V. sativa* as a human food source, it is important to understand the characteristic aromas endemic to the raw seed and how processing will affect the volatile profile. Although aroma characteristics have been well documented in other commercial legume species such as soybean and lentil, there is no such information regarding *V. sativa*. Typical of leguminous crops are inherent beany or green off-flavors that tend to increase and develop following harvesting, processing and storage (Roland et al., 2017). It is known that processing can accelerate oxidative pathways, increase enzyme activity, and cause the natural breakdown of lipids (Noordraven et al., 2021), whilst some processing methods generate volatiles capable of masking and modifying undesirable aroma volatiles, potentially improving consumer acceptability (Schindler et al., 2011)). Little is known about how processing would affect the volatile profile of *V. sativa* and whether processing would have a significant effect on palatability.

The aim of developing *V. sativa* as a human food crop will require a detailed understanding of how complex flavors can be developed through standard and more innovative processing routes. This work therefore aims to explain flavor development in *V. sativa* and the effect of hull removal, overnight soaking, germination and microwave cooking on aroma properties, in addition to fermentation by *Bacillus subtilis* (natto) and *Rhizopus oligosporus* (tempeh) for both *V. sativa* and *Vicia villosa* (hairy vetch).

2. Materials and methods

2.1. Raw materials

Seeds from two *V. sativa* varieties (Jose and Amelia) along with a *V. villosa* variety (Latigo) were procured from Kings Crops (Diss, UK) from plants grown on a large commercial scale in Southern Europe, under natural condition in Spanish fields. Seeds from a wild variety of *V. sativa* known as Segetalis were obtained from Emorgate Seed (Tilney All Saints, UK) from plants grown in Norfolk, UK.

2.2. Food processing methodology

2.2.1. De-hulling of vetch seed

A rice mill (NW1000 Turbodomestic, Bang Nam Priao, Thailand) was used to separate vetch endosperm and hull. In brief, threshed seed was fed at 200 g/min into the hopper and passed between two sets of rubber rollers set to approximately 1 mm of separation to physically split and shatter the whole seed. The endosperm and hull were separated using the white rice setting. The de-hulled seeds were then sieved using a sieve shaker (OCTAGON Digital, London, UK) fitted with a 2 mm nickel sieves (Fisher Scientific, Loughborough, UK), therefore > 2 mm de-hulled seed were collected for further processing.

2.2.2. Soaking of vetch

All de-hulled and whole vetch seed were washed with 9 °C running tap water until the water ran clear. Seeds were soaked in 200 g batches in 500 mL of room temperature water for 24 h. The soaked seeds were drained and then rinsed again with 9 °C running tap water until the water ran clear, this occurred immediately prior to each treatment outlined below. All treatments were performed in triplicate.

2.2.3. Germination of vetch

Previously soaked seed (200 g) was air dried at 21 °C for 8 h before being placed on a sieve and allowed to germinate. The seeds were

soaked briefly for 10 s in water with light agitation each day to maintain adequate moisture for seed gemination. The germination happened in natural conditions (spring, northern hemisphere). After eight days, the seeds were dried in a dehydrator (L'equip Filterpro, Salt Lake City, UT, USA) at 50 °C until seed mass remained constant. Dehydrated seeds were milled to a fine powder using a coffee grinder (DēLonghi KG49, Via L. Seitz, Italy) and stored at 17 °C in an airtight container along with 50 g of silica gel (Sigma Aldrich, St Louis, MO, USA).

2.2.4. Microwave treatment for vetch

Samples of soaked vetch seed (15 g) were spread as a single layer within a lidded Pyrex® 80 mm sterile diameter petri dish and placed in the center of an 800 W microwave (Cookworks Signature EG820CPT, Milton Keynes, UK). The seed was microwaved in 30 s increments for 2.5 min. Once microwaved, the seeds were cooled, before being frozen at −20 °C for storage.

2.2.5. Natto production from vetch

Samples of soaked vetch seed (200 g) were placed on a foil tray inside a pressure steamer (Prestige High Dome 6L, Wirral, UK) and steamed for 40 min. In a sterilized bowl, 20 mg of *B. subtilis* spores (Nattomoto, Yuzo Takahashi Laboratory CO, Japan) were added to 10 mL of 80 °C tap water to initiate spore activation. Upon cooling of the steamed vetch (to 25 °C), the *B. subtilis* solution was added and mixed thoroughly. The inoculated seed was transferred to a flat tray and a double layer of perforated cling film applied; one layer was pressed against the surface of the vetch, while the other was applied taut to seal the tray. The natto was fermented (Binder Standard Incubator BD 400, Bohemia, NY, USA) at 37 °C for 20 h under humid conditions. The resulting natto samples were frozen at −20 °C for storage.

2.2.6. Tempeh production from vetch

Samples of soaked vetch seed (200 g) were placed in a 1 L saucepan (Ready Steady Cook, Imperial, Whetstone, UK) and covered with 500 mL of 9 °C tap water supplemented with 25 mL of white wine vinegar (Sainsbury's, London, UK) to achieve a pH of 3.6 (Hanna Precision pH 209, Leighton Buzzard, UK). The seed was boiled for 30 min, after which the cooking liquid was drained. The boiled seed was spread on a baking tray to cool (to 35 °C) and 2.5 g of *R. oligosporus* spores (Aneka Fermentasi Industri, Padalarang, Indonesia) were added and mixed thoroughly. The inoculated seed was poured and sealed inside perforated zip lock bags (15 × 9 cm) with light pressure applied to aid compaction. The tempeh-vetch mixture was incubated (Binder Standard Incubator BD 400, Bohemia, NY, USA.) at 30 °C for 72 h, before being frozen at −20 °C for storage.

2.2.7. Freeze-drying of samples

Samples were spread as thin layers within foil containers before being frozen in a −80 °C freezer (New Brunswick Scientific U410, Edison, NJ, USA) for a minimum of 8 h. Samples were freeze dried (Edwards Modulgo, Chorley, UK) until their mass remained unchanged. Freeze-drying was undertaken to increase the gas phase volume to facilitate transfer of compounds to the headspace, with rapid sublimation of the liquid phase limiting reactions of heat libel volatiles following seed processing. Freeze-dried samples were subsequently milled to a fine powder using a coffee grinder (DēLonghi KG49, Via L. Seitz, Italy) and stored at 17 °C in an airtight container along with 50 g of silica gel (Sigma Aldrich, St Louis, MO, USA).

2.3. Headspace solid phase microextraction gas chromatorgpahy – Mass spectrometry analysis

Headspace solid-phase microextraction (SPME) GC-MS analysis was performed on a Trace 1300 series gas chromatograph (Thermo Fisher Scientific, Hemel Hempstead, UK) to determine the volatile profiles of the different vetch samples. An SPME fiber 2 cm Supelco DVB/CAR/

PDMS, (Sigma Aldrich, St Louis, MO, USA) was exposed to a vial containing freeze-dried vetch powder (2 g) for 15 min at 70 °C, with intermittent agitation. The fiber was then desorbed in the GC inlet at 250 °C for 1 min. Separation was carried out on a 30 m ZB WAX capillary GC column (0.25 mm ID × 1 μm Df, Phenomenex, Torrance, CA, USA). The GC oven temperature was initially 40 °C for 2 min, then increased to 240 °C at a rate of 6 °C/min, this temperature for 5 min. Helium was used as the carrier gas and pressure was kept constant at 18 psi. Full scan mode was used to detect the volatile compounds and the MS scan range was m/z 35 to 300 for the duration of each run. Samples were analyzed in triplicate. Volatiles were identified by comparing their mass spectra against reference libraries (NIST/SPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, Columbia, MO, USA) and with linear retention index (LRI) from the literature, confirmed with the use of alkane standards (C7 to C40, Sigma Aldrich, St Louis, MO, USA). Quantification of volatiles was performed through a comparison of integrated peak areas of individual alkane standards (40 mg/kg $^{-1}$) with those compounds of interest possessing the closest retention times, from this relative concentration of the compound was extrapolated. GC–MS data was initially processed with Xcalibur™ software (Thermo Fisher Scientific). Chemometric analysis consisted of principal component analysis (PCA) following the systematic removal of compounds that were not found to be significant after two-way ANOVA ($p > 0.05$) followed by Dunnett's multiple comparisons test (GraphPad Software version 9.3.1, San Diego, CA, USA). Subsequent compounds were removed if found to contain no sensory descriptors within the GoodScents Company information system (Oak Creek, WI, USA). All PCAs and dendrograms were generated with XLStat Sensory 2019 (Addinsoft, New York, NY, USA). For hierarchical clustering and construction of a heatmap of the correlation matrix the data was normalized to the lowest and greatest relative concentrations across all processed vetch samples for each individual volatile compound detected. Pearson correlation coefficient was computed to assess the correlations between different volatile compounds. (GraphPad Software version 9.3.1, San Diego, CA, USA).

2.4. Whole genome sequencing and DNA library preparation

To prepare *Vicia* whole genome sequencing (WGS) short-read data, *Vicia* seeds were sterilized and germinated on wet filter paper at 21 °C for 48 h. Germinated seeds were grown in duplicate using a paper roll method previously published (Jansen et al., 2013). After five days of growth, leaf tissue (two leaves per sample) and root tissue (5 cm from root tip) were harvested and snap-frozen in liquid nitrogen. DNA was extracted using a modified CTAB protocol (Porebski et al., 1997). Illumina library preparation with an insert size of 300 bp and pair-end (2x150 nt) sequencing was performed on a Novo-Seq 6000 (Illumina, RRID:SCR_016387, Novagene Co. Ltd., Singapore), with 40–50 GB of sequence data obtained per library.

2.5. Phylogenetic analysis

Illumina paired-end reads of *V. sativa* (ATC60338, ATC62022, IR28, Amelia, Jose, Segetalis), *Vicia. pannocia* (K33208), *Vicia. narbonensis* (ATC65521), *Vicia. ervilia* (ATC64812), and *V. villosa* (Latigo) were trimmed for adapters and low-quality reads (Phred score < 20) using Trim Galore! v0.6.7 (Trim Galore, RRID:SCR_011847). High quality reads were then mapped to the *V. sativa* genome (Xi et al., 2021) using BWA mem v0.7.17 (BWA, RRID:SCR_010910) (Li & Durbin, 2009) with default parameters. The mapping result was converted into BAM file format using Samtools v1.13 (SAMTOOLS, RRID:SCR_002105) (Li et al., 2009). The duplicated reads from the BAM files were marked by Picard v2.26.3 (Picard, RRID:SCR_006525). For each BAM file, GATK v4.2.2.0 (GATK, RRID:SCR_001876) (Depristo et al., 2011) HaplotypeCaller module was used to generate a variant calling file (.gvcf file) and the joint variant calling was performed using GenomicsDBImport and

GenotypeGVCFs GATK modules. The produced variant calling files were then filtered using a GATK VariantFiltration module with the SNP filter-expression parameters set as follows: Qual By Depth (QD) < 2.0, RMS Mapping Quality (MQ) < 40.0, Fisher Strand (FS) > 60.0, Strand Odds Ratio (SOR) > 3.0, Mapping Quality Rank Sum Test (MQRankSum) < -12.5, ReadPositionRankSum < -8.0. The Indel filter-expression parameters were set as follows: QD < 2.0, FS > 200.0, SOR > 10.0, MQRankSum < -12.5 and ReadPositionRankSum < -8.0.

The filtered variants for each species were analysed using principal components analysis (PCA) from Plink v1.90b6.21 (PLINK, RRID:SCR_001757) (Purcell et al., 2007) and the first three eigenvectors were plotted. A maximum likelihood tree was constructed by using SNPhylo v 20,180,901 (Lee et al., 2014) in which parameters were set as follows: multiple alignment was performed by MUSCLE, LD threshold = 0.1, the number of the last autosome = 9 and bootstrap value = 1000.

3. Results and discussion

3.1. Classification confirmation of chosen Vicia varieties by phylogenetic analysis utilizing SNP data

Phylogenetic analysis was performed to confirm the classification of those varieties used in this study. Whole genome sequencing (WGS) reads of five *V. sativa* varieties, and four additional *Vicia* species, V. *villosa* variety Latigo, *V. pannocia*, *V. ervilla* and *V. narbonensis* were generated and aligned to the *V. sativa* reference genome (Xi et al., 2021). Single nucleotide polymorphisms (SNPs) were identified and used to determine the genetic relationship amongst the genomes by phylogenetic analysis. Phylogenetic analysis showed variety Segetalis to be closely related to *V. sativa* (Fig. 1b). PCA (Fig. 1d) further supported that variety Segetalis was *V. sativa*. In the group of *V. sativa*, variety Segetalis was found to be genetically divergent from varieties Amelia and Jose.

3.2. Inter- and intra-species variation between volatile profiles of raw vetch seed

Forty-six distinct volatile aroma compounds were identified by headspace SPME GC-MS analysis of raw seed from four *Vicia* varieties (Table 1); 17, 15, 27 and 18 individual volatile compounds were detected in the *V. sativa* commercial varieties Amelia and Jose, the landrace Segetalis, and the *V. villosa* variety Latigo, respectively. The lowest total relative concentrations were observed in the commercial *V. sativa* varieties, Amelia and Jose (Table 1), with no significant differences in total relative concentration being observed between the two. The commercial *V. villosa* variety, Latigo demonstrated significantly higher ($p < 0.05$) total relative concentrations than that of both commercial *V. sativa* varieties, Amelia and Jose. The wild *V. sativa* variety Segetalis possessed a total relative concentration more than three times higher than that of any other seed sample analyzed. The observed phenotypic variation in total aroma volatiles between the commercial and wild varieties of *V. sativa* potentially indicate a wide genetic basis for differing levels of volatile abundance and with further study could inform future breeding programs.

Proportions of individual compound classes showed significant interspecies differences between the samples of *V. sativa* and *V. villosa,* as well as significant intraspecies differences within *V. sativa* between the commercial varieties, and the wild Segetalis. Between the two commercial varieties of *V. sativa*, no significant differences in the proportions of individual compound classes were observed; the primary chemical group, furans, typically accounted for 25% of the total relative concentration, followed by alcohols (20.6%), ketones (7.6%), carboxylic acids (3.0%), aldehydes (2.7%) and alkenes (0.6%). Commercial *V. sativa* 'Amelia' and 'Jose' were found to be most similar in both flavor analysis (Fig. 1a, c) and genetic analysis (Fig. 1b, d). The wild variety Segetalis had some significant differences in the proportions of compound classes when compared to the commercial varieties. Relative
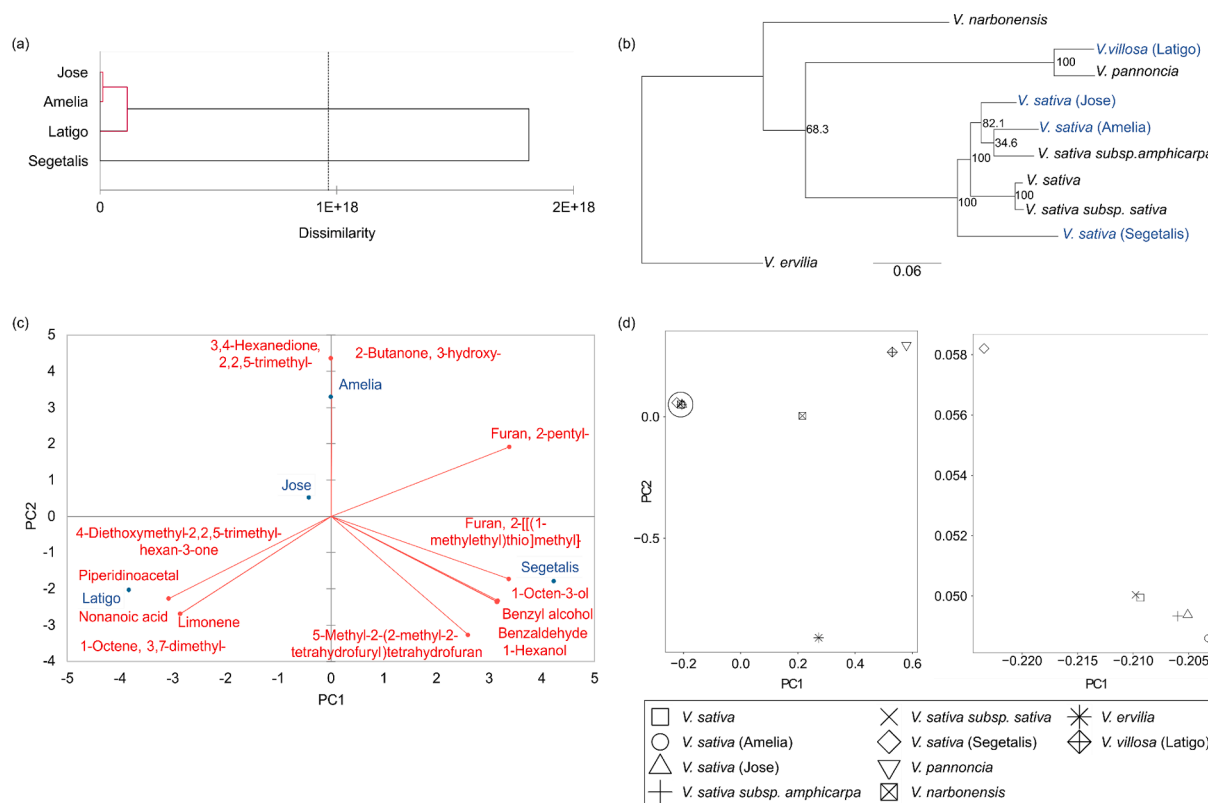
Fig. 1. (a) Agglomerative hierarchical clustering (AHC) indicating the dissimilarity between overall volatiles across analysed samples. (b) Maximum likelihood phylogenetic tree (1000 bootstrap) inferred from the whole genome SNPs of six *V. sativa* varieties, Amelia, Jose, Segetalis, Studenica (reference genome is labelled as *V. sativa*), *V. sativa subsp amphicarpa*, *V. sativa subsp sativa*, *V. villosa* variety Latigo, V. *pannoncia, V. ervilla* and *V. narbonensis*. Variety Segetalis is similar to other analysed *V. sativa* accessions. (c) Principal components analysis (PCA) on the static headspace data (averaged) obtained by SPME GC-MS analysis of the untreated seed from three *V. sativa* varieties Amelia, Jose and Segetalis and a *V. villosa* variety Latigo. PC1 and PC2 account for 58% and 32% of the variance respectively. (d) Principal components analysis (PCA) of the genetic similarity amongst the 10 sequenced *Vicia* genomes. Six *V. sativa* varieties Amelia, Jose, Segetalis, *subsp.* amphicarpa and *subsp. sativa.* PC1 and PC2 account for 22.7% and 20% of the variance respectively. Right panel represents a zoomed focus on the *V. sativa* grouped and highlighted within the circle of the left panel.

quantities of aldehydes (18%) were found to be higher in Segetalis than the two commercial *V. sativa* varieties, whilst proportions of furans were significantly less in Segetalis (10%). Segetalis also had some significant deviations from Amelia specifically, with higher proportions of alcohols (28%) and ketones (0.2%).

The classes of individual volatile compounds and their proportions within *V. villosa* Latigo were found to be significantly different from all *V. sativa* samples analyzed. The primary group of compounds detected in *V. villosa* were that of terpenes, which represented 12% of total relative concentration, followed by alkenes (10%), aldehydes (9.8%), alcohols (8%), ketones (6%), carboxylic acids (3%), and pyrazines (1%). The abundance of terpenes was primarily due to the presence of limonene, a botanical volatile which occurs naturally in higher plants. Within legumes it has been identified as a potential ovipositional deterrent to *Callosobruchus maculatus* beetles feeding on black eyed beans (Hudaib et al., 2010) and the isolated substance is a commonly used component within commercial insecticides (Hollingsworth, 2005).

The most dominant aroma compounds commonly detected amongst all *V. sativa* samples were that of 2-pentyl furan (29%), benzyl alcohol (12.7%), benzaldehyde (10%), 1-octen-3-ol (9.4%) and 1-hexanol (7.9%). Collectively these five compounds accounted for an average of 80% of the total relative concentration and are all documented as producing off-flavors within other pulse species (Kouris-Blazos & Belski, 2016). Formation of 2-pentyl furan occurs during lipid oxidation and degradation, observed consistently in legumes following long-term storage (Kettlitz et al., 2019) and is associated with higher levels of biological activity within the raw seed. Alcohols were consistently amongst the most abundant compound classes detected in the headspace

of samples, particularly the volatiles of 1-octen-3-ol (7.5%), 2-isopropyl-5-methyl-1-heptanol (5%) and phenylethyl alcohol (1.2%). The presence of 1-octen-3-ol has been observed in other leguminous species such as chickpea and soybean and is characterized by a mushroom, earthy odor ((Khrisanapant, Kebede, & Leong, 2019). The generation of alcohols are likely to have occurred following the enzymatic breakdown of seed lipids post-harvest; initial hydroperoxidation of polyunsaturated fatty acids by lipoxygenases (Roland et al., 2017) would be followed by further enzymatic activity in which alcohol dehydrogenases catalyze the newly produced aldehydes to subsequent alcohols (Gomes et al., 1982). Furthermore, 2-pentyl furan and 1-octen-3-ol have relatively low odor thresholds compared to benzyl alcohol and benzaldehyde which may indicate that they are of high importance for *V. sativa*.

3.3. Effect of different processing methods on the volatile profile of common and hairy vetch

Seed processing methods were separated into two distinct categories: (i) dehulling and soaking under typical post-harvest processing, which are classed as those processes typically performed on the raw seed prior to commercial sale as whole beans/legumes; and (ii) germination, microwaving, and natto and tempeh fermentation, methods which are classed as advanced processing methods. Overall, the various processes increased the number of volatile compounds detected to 95 identified volatile aroma compounds (Table 2). The different forms of Amelia, Jose and Latigo contained 57, 68 and 63 unique aroma volatiles, respectively.

Table 1

Volatile compounds identified in *V. sativa* varieties Amelia, Jose and Segetalis and *V. villosa* variety Latigo using HS-SPME-GC-MS. Data represents the mean relative concentration (mg/kg^{-1}) ± standard deviations (n = 3) of volatiles, -: not detected.

Compounds	Amelia	Jose	Segetalis	Latigo	RI
	Vicia sativa			*Vicia villosa*	
Alcohols					
1,6-Octadien-3-ol, 3,7-dimethyl-	–	–	–	2.6 ± 0.1	1555
1-Hexanol	–	7.2 ± 0.1	14.3 ± 0.6	4.0 ± 0.2	1363
1-Hexanol, 2-ethyl-	–	–	1.0 ± 1.0	1.0 ± 0.8	1497
1-Nonanol	–	–	1.1 ± 0.2	–	1667
1-Octanol	0.5 ± 0.4	–	–	–	1566
1-Octen-3-ol	5.7 ± 0.9	4.2 ± 0.3	26.2 ± 0.9	1.8 ± 0.4	1458
2,3-Butanediol	–	1.4 ± 0.1	0.4 ± 0.1	–	1587
2,5-Dimethyl-1-hepten-4-ol	–	0.5 ± 0.4	0.8 ± 0.0	0.9 ± 0.1	1377
2-Isopropyl-5-methyl-1-heptanol	4.5 ± 1.0	2.9 ± 0.5	4.4 ± 0.2	3.7 ± 0.4	1322
Benzyl alcohol	1.1 ± 0.2	0.8 ± 0.2	122 ± 9.6	–	1897
Phenylethyl Alcohol	0.6 ± 0.5	0.4 ± 0.0	1.0 ± 0.3	2.4 ± 0.3	1935
α-Terpineol	–	–	0.3 ± 0.1	–	1714
SubTotal (mg/kg^{-1})	12.4 ± 3.0	17.4 ± 1.6	171 ± 13.0	16.4 ± 2.3	
Aldehydes					
Acetaldehyde	–	–	–	0.2 ± 0.0	690
Benzaldehyde	–	0.2 ± 0.2	106 ± 14.8	–	1558
Nonanal	2.9 ± 0.8	1.6 ± 1.1	3.3 ± 1.1	2.5 ± 1.2	1411
Octanal dimethyl acetal	0.3 ± 0.3	–	–	–	1635
Piperidinoacetal	–	–	–	16.8 ± 4.5	1047
SubTotal (mg/kg^{-1})	3.2 ± 1.1	1.8 ± 1.3	109 ± 15.9	19.5 ± 5.7	
Alkenes					
1-Octene, 3,7-dimethyl-	–	–	–	21.6 ± 2.2	1102
Caryophyllene	–	–	2.8 ± 0.2	–	1623
SubTotal (mg/kg^{-1})	–	–	2.8 ± 0.2	21.6 ± 2.2	
Amines					
Cedrene-V6	0.7 ± 0.6	–	–	–	1593
SubTotal (mg/kg^{-1})	0.7 ± 0.6	–	–	–	
Carboxylic acids					
2-Propanamine, 2-methyl-	0.9 ± 0.8	–	–	–	926
2-Butenedioic acid, 2,3-dimethyl-, dimethyl ester	–	–	0.9 ± 0.5	–	1119
Acetic acid	–	–	–	–	1474
Benzoic acid, ethyl ester	–	–	0.8 ± 0.1	–	1694
Benzoic acid, methyl ester	0.4 ± 0.4	0.5 ± 0.4	3.9 ± 0.3	0.7 ± 0.1	1653
Butanoic acid, 2-ethyl-, 1,2,3-propanetriyl ester	–	–	0.2 ± 0.2	–	1724
Butanoic acid, 3-methyl-	–	0.2 ± 0.2	2.5 ± 0.3	–	1684
Heptanoic acid	–	–	0.6 ± 0.3	–	1966
Hexanoic acid	–	–	1.1 ± 1.0	–	1859
Nonanoic acid	–	2.8 ± 2.1	–	4.3 ± 4.2	2174
Pentanoic acid	–	–	0.4 ± 0.0	–	1755
SubTotal (mg/kg^{-1})	1.3 ± 1.1	3.4 ± 2.6	10.3 ± 2.6	5.0 ± 4.3	1524
Furans					
Pentanoic acid, 2-hydroxy-, methyl ester	–	–	–	0.7 ± 0.2	
5-Methyl-2-(2-methyl-2-tetrahydrofuryl) tetrahydrofuran	–	–	26.8 ± 4.1	–	994
Furan, 2-[[(1-methylethyl)thio]methyl]-	–	–	4.8 ± 3.8	–	1094
Furan, 2-pentyl-	24.9 ± 2.6	15.4 ± 8.6	29.4 ± 2.2	–	1245
SubTotal (mg/kg^{-1})	26.2 ± 3.7	21.6 ± 13.3	73.5 ± 13.9	10.0 ± 8.7	
Ketones					
2(3H)-Furanone, 5-ethyldihydro-	–	–	1.1 ± 0.2	–	1740
2-Butanone	–	0.3 ± 0.2	–	–	915
2-Butanone, 3-hydroxy-	9.7 ± 0.9	–	–	–	1308
3,4-Hexanedione, 2,2,5-trimethyl-	4.9 ± 4.2	–	–	–	1092
4-Diethoxymethyl-2,2,5-trimethyl-hexan-3-one	–	–	–	11.4 ± 1.5	1354
Ethanone, 1-(2-furanyl)-	0.4 ± 0.4	0.3 ± 0.1	–	1.2 ± 0.1	1374
SubTotal (mg/kg^{-1})	15.0 ± 5.5	0.6 ± 0.3	1.1 ± 0.2	12.6 ± 1.6	
Pyrazines					
Pyrazine, 3-ethyl-2,5-dimethyl-	0.3 ± 0.3	–	–	–	1469
Pyrazine, trimethyl-	1.4 ± 0.7	–	–	2.6 ± 0.7	1428
SubTotal (mg/kg^{-1})	1.7 ± 1.0	–	–	2.6 ± 0.7	
Pyrazine Deriviatives					
Pyridine, 2-tridecyl-	–	–	0.7 ± 0.6	–	1182
SubTotal (mg/kg^{-1})	–	–	0.7 ± 0.6	–	
Sulfides					
Dimethyl sulfide	–	–	0.8 ± 0.0	–	768
SubTotal (mg/kg^{-1})	–	–	0.8 ± 0.0	–	
Sulfides					
Limonene	–	–	–	24.9 ± 1.6	1214
SubTotal (mg/kg^{-1})	–	–	–	24.9 ± 1.6	
Total (mg/kg^{-1})	60.5 ± 16.1	44.8 ± 19.1	369 ± 46.4	87.7 ± 25.6	

3.3.1. Dehulling

The mechanical removal of the outer hull had no significant effect on the aroma volatile total relative concentration compared to the control raw seed (Fig. 2a). The proportions of furans (17%), alcohols (15%), aldehydes (7%), alkenes (2.3%) and carboxylic acids (1.4%) were significantly less than the raw seed controls, whilst esters (22%) and pyrroles (8%) were newly identified in the dehulled samples, after being entirely absent in raw samples. The reduction of alcohols following dehulling has been previously observed in Faba bean (Akkad, Kharraz, Han, House, & Curtis, 2019), where increased exposure of the endosperm to environmental oxygen, encouraged partial oxidation of the existing alcohols into subsequent aromatic compounds. The decline of both alcohols and carboxylic acids whilst coinciding with significant increases in esters is characteristic of esterification reactions (Otera & Nishikido, 2009). These processes likely caused production of 3-methyl-pentan-3-yl propyl carbonate in Amelia and dimethyl ethylidenemalonate in Jose and Latigo, both compounds characterized as having 'fruity' aromas.

Table 2

Volatile compounds identified in *V. sativa* (varieties Amelia and Jose) and *V. villosa* (Latigo) following different processing methods (dehulled, soaked, germinated, microwaved, natto fermentation, tempeh fermentation.) utilizing HS-SPME-GC-MS. Data represents the mean relative volatile concentration (mg/kg^{-1}) ± standard deviations (n = 3), -: not detected.

Compounds	Dehulled Amelia	Dehulled Jose	Dehulled Latigo	Soaked Amelia	Soaked Jose	Soaked Latigo	Germinated Amelia	Germinated Jose	Germinated Latigo	Microwaved Amelia	Microwaved Jose	Microwaved Latigo	Natto Amelia	Natto Jose	Natto Latigo	Tempeh Amelia	Tempeh Jose	Tempeh Latigo	RI
Alcohols																			
1,6-Octadien-3-ol, 3,7-dimethyl-	–	–	2.9 ± 0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1555
1-Butanol, 3-methyl-'	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	58.1 ± 13.2	309 ± 77.4	65.4 ± 5.9	1218
1-Heptanol	–	–	–	–	–	1.6 ± 1.3	–	–	–	–	–	–	–	–	–	–	–	–	1464
1-Hexanol	–	5.6 ± 1.0	3.5 ± 0.3	–	3.2 ± 0.3	22.6 ± 12.8	–	3.1 ± 0.8	5.8 ± 0.8	–	–	–	–	–	0.4 ± 0.1	–	2.4 ± 0.8	1.0 ± 0.2	1363
1-Hexanol, 2-ethyl-	–	–	1.8 ± 0.3	–	1.4 ± 0.2	5.5 ± 2.2	–	–	–	–	0.9 ± 0.2	0.4 ± 0.1	–	–	11.7 ± 10.4	–	–	2.9 ± 0.3	1497
1-Nonanol	–	–	–	–	–	4.8 ± 3.5	–	–	0.7 ± 0.2	–	–	–	–	–	–	–	–	–	1667
1-Octanol	0.4 ± 0.3	–	–	–	–	1.6 ± 0.9	0.8 ± 0.8	0.1 ± 0.1	1.1 ± 0.2	1.8 ± 0.2	–	–	–	–	–	–	–	–	1566
1-Octen-3-ol	5.7 ± 0.7	3.6 ± 0.9	1.0 ± 0.1	2.7 ± 0.4	2.5 ± 0.6	8.9 ± 2.8	13.5 ± 0.5	11.6 ± 1.7	17.7 ± 1.6	10.3 ± 0.4	4.2 ± 0.4	4.7 ± 1.0	6.1 ± 0.2	7.5 ± 2.4	3.2 ± 0.6	–	–	1.1 ± 1.0	1458
2,3-Butanediol	–	1.3 ± 0.2	–	–	–	–	0.8 ± 0.7	0.3 ± 0.0	–	–	–	–	6.4 ± 5.6	–	–	133 ± 19.4	182 ± 53.6	18.3 ± 4.4	1587
2,5-Dimethyl-1-hepten-4-ol	–	0.3 ± 0.3	0.4 ± 0.2	–	0.9 ± 0.o0	3.2 ± 1.4	–	2.8 ± 0.2	2.9 ± 0.2	–	0.6 ± 0.5	1.0 ± 0.2	–	1.8 ± 1.6	1.3 ± 0.1	–	–	0.4 ± 0.1	1377
2-Furanmethanol	–	–	–	–	–	–	–	–	–	–	2.9 ± 0.6	–	–	–	–	549 ± 113	1017 ± 221	378 ± 19.5	1322
2-Isopropyl-5-methyl-1-heptanol	1.7 ± 0.1	1.4 ± 0.2	2.2 ± 0.2	2.6 ± 0.5	1.8 ± 0.1	3.5 ± 0.5	3.9 ± 1.9	3.0 ± 0.4	3.3 ± 0.9	4.3 ± 0.9	2.1 ± 0.1	2.3 ± 0.4	1.9 ± 1.7	4.1 ± 0.2	2.8 ± 0.7	2.6 ± 0.6	4.3 ± 2.2	2.5 ± 0.4	1897
Benzyl alcohol	–	0.3 ± 0.4	–	–	–	0.6 ± 0.5	–	–	0.2 ± 0.2	–	–	–	1 ± 0.3	3.1 ± 3.1	–	4.4 ± 0.4	9.4 ± 3.1	–	946
Ethanol	–	–	–	114 ± 7.0	29.4 ± 5.0	66.5 ± 9.8	74.8 ± 10.5	–	–	–	–	–	72.5 ± 28	23.4 ± 18.0	43.5 ± 17.3	345 ± 78.5	447 ± 63.9	283 ± 7.0	1935
Phenylethyl Alcohol	0.5 ± 0.2	0.4 ± 0.1	1.7 ± 1.5	0.5 ± 0.1	0.3 ± 0.1	1.2 ± 0.8	–	–	0.2 ± 0.2	–	–	–	0.5 ± 0.1	1.2 ± 0.5	0.6 ± 0.1	5.2 ± 0.3	63.1 ± 20.1	3.2 ± 0.3	1674
SubTotal (mg/kg^{-1})	8.2 ± 1.4	13.0 ± 3.0	13.6 ± 2.6	120 ± 8.0	39.5 ± 6.4	120 ± 36.5	93.7 ± 14.3	20.9 ± 3.2	31.8 ± 4.1	16.4 ± 1.5	10.7 ± 1.8	8.4 ± 1.7	88.5 ± 35.8	41.1 ± 25.8	63.5 ± 29.3	–	–	–	
Aldehydes																			
Acetaldehyde	–	–	–	–	–	3.1 ± 1.9	1.0 ± 0.9	–	–	0.5 ± 0.5	–	–	0.4 ± 0.4	1.1 ± 0.9	0.5 ± 0.2	3.3 ± 1.0	5.6 ± 3.4	1.2 ± 0.1	690
Benzaldehyde	0.5 ± 0.7	–	–	–	–	1.9 ± 1.7	1.8 ± 0.5	1.4 ± 0.1	1.7 ± 0.3	–	–	–	–	3.4 ± 2.6	–	10.8 ± 2.4	–	–	1558
Butanal, 2-methyl-	–	–	–	–	–	–	–	2.8 ± 0.3	1.6 ± 0.4	–	–	–	–	–	–	–	–	–	924
Butanal, 3-methyl-	–	–	–	–	–	–	–	5.4 ± 0.5	2.9 ± 0.6	0.7 ± 0.8	–	–	–	–	–	–	–	–	928
Hexanal	–	–	–	–	–	–	–	43.8 ± 2.6	–	–	43.5 ± 5	–	–	–	26.0 ± 2.4	–	–	20.7 ± 1.0	1098
Nonanal	1.3 ± 0.1	0.4 ± 0.4	–	1.4 ± 0.2	–	2.7 ± 0.5	14.9 ± 1.2	6.5 ± 0.2	19.8 ± 3.4	4.3 ± 0.1	–	1.4 ± 0.8	1.6 ± 0.2	–	1.9 ± 0.1	1.5 ± 1.3	2.0 ± 0.7	1.0 ± 0.9	1411
Octanal dimethyl acetal	–	–	–	–	–	–	–	–	–	0.6 ± 0.1	–	–	–	–	–	–	–	–	1635
Pentanal	–	–	–	–	–	–	5.2 ± 2.8	–	–	–	–	–	–	2.1 ± 0.6	–	–	–	–	929
Piperidinoacetal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1047

6

Table 2 (*continued*)

Compounds	Dehulled Amelia	Jose	Latigo	Soaked Amelia	Jose	Latigo	Germinated Amelia	Jose	Latigo	Microwaved Amelia	Jose	Latigo	Natto Fermentation Amelia	Jose	Latigo	Tempeh Fermentation Amelia	Jose	Latigo	RI
	–	–	18.3 ± 4.8	28.7 ± 1.3						35.6 ± 0.4		20.2 ± 0.6	35.6 ± 8.8			27.8 ± 8.9			
Propanal, 2-methyl-	–	–	–	–	–	–	–	0.7 ± 0.6	–	–	–	–	–	–	–	–	–	–	820
SubTotal (mg/kg-1)	1.8 ± 0.7	0.4 ± 0.4	18.3 ± 4.8	30.1 ± 1.5	–	7.8 ± 4.2	23.0 ± 5.4	16.7 ± 1.8	69.9 ± 7.4	41.7 ± 1.8	43.5 ± 5	21.6 ± 1.4	37.6 ± 9.3	6.6 ± 4.2	28.4 ± 2.8	43.3 ± 13.6	7.6 ± 4.1	23.0 ± 2.0	
Alkenes																			
1,1′-Ethylene-2,2′-bipyridyldiylium	–	–	6.9 ± 3.6	–	7.2 ± 3.3	–	–	–	–	–	–	–	–	–	–	–	–	–	1093
Caryophyllene	–	–	–	0.6 ± 0.0	0.4 ± 0.1	0.4 ± 0.5	–	–	–	0.6 ± 0.5	–	–	0.6 ± 0.5	5.1 ± 5.8	0.5 ± 0.5	21.7 ± 1.1	43.3 ± 6.7	10.3 ± 0.7	1102
Ethoxyacetylene	–	–	–	–	–	–	10.9 ± 1.6	–	–	–	–	–	–	–	–	–	–	–	1623
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2 ± 0.2	–	–	1593
α-Guaiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2 ± 0.2	0.8 ± 0.7	1265
SubTotal (mg/kg⁻¹)	–	–	6.9 ± 3.6	0.6 ± 0.0	7.6 ± 3.4	0.4 ± 0.5	10.9 ± 1.6	–	–	0.6 ± 0.5	–	–	0.6 ± 0.5	5.1 ± 5.8	0.5 ± 0.5	21.9 ± 1.3	43.5 ± 6.9	11.1 ± 1.4	
Amines																			
2-Propanamine, 2-methyl-	–	–	–	–	–	–	3.3 ± 0.5	–	–	–	–	–	–	–	–	–	–	–	926
2-Pyridinamine, 3,6-dimethyl-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.5 ± 2.2	–	–	1408
Octadecylamine	–	8.2 ± 9.6	14.6 ± 5.3	–	–	–	–	19.9 ± 8.4	21.2 ± 6.7	–	–	–	–	24.8 ± 12.5	–	–	–	–	1208
SubTotal (mg/kg⁻¹)	–	8.2 ± 9.6	14.6 ± 5.3	–	–	–	3.3 ± 0.5	19.9 ± 8.4	21.2 ± 6.7	–	–	–	–	24.8 ± 12.5	–	2.5 ± 2.2	–	–	
Carboxylic acids																			
2-Butenedioic acid, 2,3-dimethyl-, dimethyl ester	–	–	–	–	–	–	–	0.5 ± 0.4	–	–	0.7 ± 0.7	–	–	0.5 ± 0.5	–	–	–	–	1119
2-Propenoic acid, 2-methyl-, octyl ester	–	–	–	–	–	–	11.9 ± 1.6	–	–	11.0 ± 9.6	–	–	5.7 ± 5.0	–	–	–	–	–	1111
Acetic acid	0.9 ± 0.2	–	–	–	0.3 ± 0.3	–	30.0 ± 1.4	2.6 ± 0.3	1.8 ± 0.6	–	0.6 ± 0.6	–	3.1 ± 2.7	4.8 ± 4.5	8.9 ± 2.4	3.9 ± 0.9	12.3 ± 5.9	–	1474
Benzoic acid, ethyl ester	–	–	–	–	0.8 ± 0.7	1.1 ± 0.7	–	–	–	–	–	–	–	–	–	3.7 ± 1.0	10.8 ± 5.1	1.0 ± 0.2	1694
Benzoic acid, methyl ester	–	0.4 ± 0.2	0.7 ± 0.1	–	–	–	–	–	0.3 ± 0.0	0.6 ± 0.5	0.3 ± 0.1	–	–	–	–	28.8 ± 4.8	–	–	1653
Butanedioic acid, dimethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	1.4 ± 0.3	0.5 ± 0.2	–	–	–	1610
Butanoic acid, 2-ethyl-, 1,2,3-propanetriyl ester	–	–	–	–	–	–	–	0.3 ± 0.1	0.6 ± 0.2	–	–	0.2 ± 0.1	–	–	–	–	–	–	1724
Butanoic acid, 2-methyl-, ethyl ester	–	–	–	–	–	–	–	–	–	6.7 ± 0.4	–	–	–	–	–	–	37.0 ± 8.7	–	1063
Butanoic acid, 3-methyl-	–	–	0.3 ± 0.1	–	–	1.5 ± 0.2	2.7 ± 0.5	2.8 ± 0.1	2.0 ± 0.2	2.0 ± 0.5	1.3 ± 0.2	–	22.8 ± 0.2	268 ± 278	3.0 ± 0.3	2.1 ± 0.5	18.0 ± 2.2	0.7 ± 0.1	1684
Decanoic acid, ethyl ester	–	–	–	–	–	8.6 ± 8.0	–	–	–	–	–	–	–	–	–	–	45.2 ± 11.0	13.6 ± 1.7	1852
Dodecanoic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4.6 ± 1.1	6.3 ± 0.3	2.4 ± 2.1	1858
Heptanoic acid	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	1966

(*continued on next page*)

Table 2 (*continued*)

Compounds	Dehulled			Soaked			Germinated			Microwaved			Natto Fermentation			Tempeh Fermentation			RI
	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	
						0.1 ± 0.2	0.7 ± 0.6		1.7 ± 0.3				0.3 ± 0.1						
Heptanoic acid, octyl ester	–	–	–	–	–	–	–	1.8 ± 1.8	1.4 ± 1.2	–	–	–	–	–	1.7 ± 1.5	–	–	–	962
Hexanoic acid	–	–	–	0.3 ± 0.3	–	–	11.0 ± 0.8	10.2 ± 0.7	12.0 ± 1.4	5.7 ± 0.3	1.4 ± 0.4	0.6 ± 0.5	2.7 ± 2.8	3.7 ± 1.9	1.7 ± 0.3	–	–	–	1859
Nonanoic acid	–	–	1.3 ± 1.3	–	0.9 ± 0.7	–	2.0 ± 1.9	3.2 ± 1.1	2.6 ± 1.7	–	1.1 ± 1.0	–	2.6 ± 1.7	3.1 ± 2.7	1.6 ± 1.5	6.6 ± 2.0	–	–	2174
Octadecanoic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.9 ± 0.3	4.7 ± 1.9	–	2458
Octanoic acid	–	–	–	–	–	0.9 ± 1.1	1.1 ± 0.5	0.7 ± 0.1	0.7 ± 0.6	–	–	–	1.2 ± 0.9	2.0 ± 1.3	0.4 ± 0.4	5.8 ± 1.4	3.0 ± 1.2	1.5 ± 0.1	2046
Octanoic acid, ethyl ester	–	–	–	3.2 ± 0.5	4.1 ± 1.0	12.5 ± 7.3	–			–	–	–	2.3 ± 0.1	2.4 ± 2.1	3.4 ± 1.7	98.5 ± 6.3	230 ± 44.8	68.6 ± 3.5	1446
Pentadecanoic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7 ± 0.2	4.4 ± 1.5	1.3 ± 0.5	2148
Pentanoic acid	–	–	–	–	–	–	–	0.3 ± 0.0	0.2 ± 0.2	–	–	–	–	–	–	–	–	–	1755
Pentanoic acid, 3,5-dioxo-, methyl ester	–	–	–	–	–	–	–	2.1 ± 0.1	–	–	–	–	–	–	–	–	–	–	1386
Propanoic acid, 2-hydroxy-, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.2 ± 1.1	–	1359
Propanoic acid, 2-methyl-	–	–	–	–	–	–	–	0.5 ± 0.0	0.2 ± 0.1	–	–	–	–	83.3 ± 89.2	0.8 ± 0.2	–	10.9 ± 8.8	–	1583
Propanoic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6.6 ± 1.0	–	969
Tetradecanoic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9.7 ± 1.2	25.0 ± 4.3	8.3 ± 2.4	2044
SubTotal (mg/kg^{-1})	0.9 ± 0.2	0.4 ± 0.2	2.3 ± 1.5	3.5 ± 0.8	6.1 ± 2.8	24.8 ± 17.5	59.4 ± 7.3	23.0 ± 4.7	25.7 ± 6.8	26.0 ± 11.3	5.4 ± 3.0	0.8 ± 0.6	40.7 ± 13.4	369 ± 380	21.9 ± 8.5	168 ± 19.6	416 ± 97.8	97.4 ± 10.7	
Esters																			
11-Tridecenyl propionate	–	–	–	–	–	–	–	–	–	0.4 ± 0.0	–	–	–	–	–	–	–	–	1641
3-Methylpentan-3-yl propyl carbonate	31.9 ± 3.7	–	–	–	–	–	51.3 ± 6.5	–	–	–	–	–	–	–	–	–	–	–	1236
Azepan-1-yl-acetic acid, methyl ester	–	–	–	–	–	–	–	–	–	0.2 ± 0.0	–	–	–	–	–	–	–	–	1647
Benzeneacetic acid, ethyl ester	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1 ± 0.1	1.6 ± 0.9	–	1808
Dimethyl ethylidenemalonate	–	9.8 ± 2.6	7.7 ± 1.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1072
Ethyl Acetate	–	–	–	7.4 ± 1.2	–	1.0 ± 0.9	–	–	–	4.5 ± 0.8	–	–	–	–	–	17 ± 1.5	79.5 ± 30.0	4.5 ± 0.8	889
SubTotal (mg/kg^{-1})	31.9 ± 3.7	9.8 ± 2.6	7.7 ± 1.5	7.4 ± 1.2	–	1.0 ± 0.9	51.3 ± 6.5	–	–	5.0 ± 0.9	–	–	–	–	–	17.1 ± 1.6	81.2 ± 31.0	4.5 ± 0.8	
Furans																			
5-Methyl-2-(2-methyl-2-tetrahydrofuryl) tetrahydrofuran	–	–	–	–	–	24.4 ± 36.8	–	–	12.6 ± 0.6	–	–	–	91.4 ± 18.3	–	–	80.4 ± 5.8	–	–	994
Furan, 2-pentyl-	14.2 ± 0.5	10.6 ± 1.1	15.7 ± 3.9	20.7 ± 2.0	16.5 ± 0.6	–	28.7 ± 0.3	26.7 ± 1.0	–	36.8 ± 0.6	31.1 ± 3.5	22.4 ± 0.9	24.3 ± 1	27.7 ± 8.9	25.3 ± 2.7	–	43.5 ± 7.5	29.6 ± 0.5	1245
Furan, tetrahydro-	–	0.3 ± 0.3	–	–	–	–	2.0 ± 0.5	0.3 ± 0.3	–	3.9 ± 0.1	–	0.2 ± 0.3	–	–	–	–	–	–	870
SubTotal (mg/kg^{-1})																			

Table 2 (*continued*)

Compounds	Dehulled Amelia	Jose	Latigo	Soaked Amelia	Jose	Latigo	Germinated Amelia	Jose	Latigo	Microwaved Amelia	Jose	Latigo	Natto Fermentation Amelia	Jose	Latigo	Tempeh Fermentation Amelia	Jose	Latigo	RI
	14.2 ± 0.5	10.9 ± 1.4	15.7 ± 3.9	20.7 ± 2.0	16.5 ± 0.6	24.4 ± 36.8	30.7 ± 0.7	27.0 ± 1.3	12.6 ± 0.6	40.7 ± 0.7	31.1 ± 3.5	22.6 ± 1.2	116 ± 19.3	27.7 ± 8.9	25.3 ± 2.7	80.4 ± 5.8	43.5 ± 7.5	29.6 ± 0.5	
Ketones																			
2-Butanone	–	0.3 ± 0.4	–	–	–	–	–	0.5 ± 0.4	–	–	3.7 ± 0.4	0.7 ± 0.6	–	1.6 ± 0.4	–	8.5 ± 1.3	–	1.2 ± 0.2	915
2-Butanone, 3-hydroxy-	1.8 ± 1.5	–	–	–	–	5.8 ± 5.4	8.6 ± 1.3	–	–	12.7 ± 1.9	–	–	14.0 ± 3.6	23.8 ± 12.6	34.1 ± 6.3	4.9 ± 1.6	–	–	1308
3,4-Hexanedione, 2,2,5-trimethyl-	15.9 ± 1.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1092
3,5-Octadien-2-one	–	–	–	–	–	3.5 ± 1.5	3.6 ± 0.2	1.8 ± 0.4	5.2 ± 0.3	18.1 ± 1.1	9.9 ± 1.0	15.0 ± 5.3	4.9 ± 0.9	8.6 ± 2.5	3.6 ± 1.0	–	10.5 ± 10.2	4.1 ± 1.4	1543
4-Diethoxymethyl-2,2,5-trimethyl-hexan-3-one	1.3 ± 0.5	–	6.7 ± 0.6	–	–	17.5 ± 6.7	–	–	17.8 ± 2.3	–	–	–	–	–	–	3.6 ± 0.5	–	–	1354
4-Hexen-3-one, 4-methyl-	1.1 ± 0.2	0.2 ± 0.2	–	0.7 ± 0.6	0.2 ± 0.2	–	–	–	–	2.1 ± 0.8	–	0.4 ± 0.3	0.5 ± 0.7	–	0.4 ± 0.4	–	–	–	838
Acetoin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2 ± 0.2	–	–	–	1572
Butyrolactone	–	–	–	–	–	–	–	–	–	–	0.5 ± 0.0	–	–	–	–	–	–	–	1670
Ethanone, 1-(2-furanyl)-	0.7 ± 0.1	0.2 ± 0.1	1.3 ± 0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2 ± 0.3	–	1374
Tetrahydrocarvone	–	–	–	–	–	–	–	–	–	–	8.1 ± 2.0	–	–	–	–	–	–	–	1091
SubTotal (mg/kg^{-1})	20.9 ± 4.1	0.7 ± 0.7	7.9 ± 0.6	0.7 ± 0.6	0.2 ± 0.2	26.9 ± 13.7	12.3 ± 1.5	2.2 ± 0.8	23.0 ± 2.6	32.9 ± 3.8	22.1 ± 3.5	16.1 ± 6.2	19.4 ± 5.2	34.0 ± 15.5	38.3 ± 7.8	17.0 ± 3.4	10.8 ± 10.5	5.3 ± 1.6	
Pyrazines																			
2,3-Dimethyl-5-ethylpyrazine	–	–	–	–	–	–	–	–	–	2.9 ± 0.1	–	–	–	–	–	–	15.0 ± 15.0	–	1482
Pyrazine, 2,5-dimethyl-	–	–	–	–	–	–	–	–	–	–	1.9 ± 0.7	–	9.4 ± 2.4	200 ± 175	–	–	–	0.2 ± 0.2	1346
Pyrazine, 3-ethyl-2,5-dimethyl-	0.3 ± 0.0	–	–	–	–	–	–	–	–	–	1.1 ± 0.3	–	0.9 ± 0.8	30.1 ± 44.3	–	–	–	–	1469
Pyrazine, tetramethyl-	–	–	–	–	–	–	–	–	–	–	–	–	–	443 ± 608	–	–	211 ± 176	–	1496
Pyrazine, trimethyl-	–	–	–	0.9 ± 0.3	4.9 ± 2	–	1.7 ± 0.2	–	1.7 ± 0.6	3.5 ± 0.1	7.5 ± 0.5	5.5 ± 1.6	12.5 ± 0.4	206 ± 286	6.1 ± 1.3	–	23.4 ± 14.6	–	1428
SubTotal (mg/kg^{-1})	0.3 ± 0.0	–	–	0.9 ± 0.3	4.9 ± 2	–	1.7 ± 0.2	–	1.7 ± 0.6	6.4 ± 0.3	10.5 ± 1.5	5.5 ± 1.6	22.8 ± 3.6	879 ± 1113	6.1 ± 1.3	–	249 ± 206	0.2 ± 0.2	
Pyrroles																			
1H-Pyrrole, 1-methyl-	–	16.5 ± 9.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1158
Indole	–	–	–	–	–	–	–	–	–	–	11.7 ± 0.2	–	–	–	5.0 ± 1.2	–	–	–	1075
SubTotal (mg/kg^{-1})	–	16.5 ± 9.8	–	–	–	–	–	–	–	–	11.7 ± 0.2	–	–	–	5.0 ± 1.2	–	–	–	
Sulfides																			
Dimethyl sulfide	–	–	–	–	–	–	76.4 ± 6.1	67.9 ± 4.5	129 ± 9.6	–	–	–	2.7 ± 2.5	–	–	–	–	–	768
SubTotal (mg/kg^{-1})	–	–	–	–	–	–	76.4 ± 6.1	67.9 ± 4.5	129 ± 9.6	–	–	–	2.7 ± 2.5	–	–	–	–	–	
Sulfoxides																			
Dimethyl Sulfoxide	–	–	–	–	–	–	–	0.3 ± 0.3	–	–	–	–	–	–	–	–	–	–	1612
SubTotal (mg/kg^{-1})	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	

Table 2 (continued)

Compounds	Dehulled			Soaked			Germinated			Microwaved			Natto Fermentation			Tempeh Fermentation			RI
	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	Amelia	Jose	Latigo	
Terpenes																			
Limonene	–	4.7 ± 5.6	18.4 ± 3.3	–	19.7 ± 0.8	–	–	0.3 ± 0.3	–	–	–	–	–	20.5 ± 3.9	–	–	–	–	1214
SubTotal (mg/kg⁻¹)	–	4.7 ± 5.6	18.4 ± 3.3	–	19.7 ± 0.8	–	–	–	–	–	–	–	–	20.5 ± 3.9	–	–	–	–	
Terpenes																			
Isothiazole, 3,4-dimethyl-	–	–	–	–	–	–	–	–	–	0.7 ± 0.6	–	–	0.4 ± 0.5	–	–	–	–	–	917
SubTotal (mg/kg⁻¹)	–	–	–	–	–	–	–	–	–	0.7 ± 0.6	–	–	0.4 ± 0.5	–	–	–	–	–	
Total (mg/kg⁻¹)	78.3 ± 10.6	64.6 ± 33.3	105 ± 27.2	184 ± 14.3	94.5 ± 16.2	205 ± 110	363 ± 44.2	178 ± 25.1	315 ± 38.4	170 ± 21.4	135 ± 18.4	75.1 ± 12.7	329 ± 90.2	1407 ± 1570	189 ± 54.0	900 ± 160	1868 ± 585	549 ± 36.7	

3.3.2. Soaking

The soaking of vetch seed significantly increased total relative concentrations, and noticeable differences in volatile aroma composition compared to the raw seed control were observed (Fig. 2). The relative proportion of alcohols compared to the untreated seed was significantly higher (50%), whilst proportions of furans (12%), aldehydes (6.8%), ketones (4.5%) and alkenes (2.4%) were significantly lower than the control. Increased alcohol content following soaking has been similarly documented in soybean (Wilkens & Lin, 1970) where high concentrations of 1-octen-3-ol were produced within six hours of initial soaking treatment (Badenhop & Wilkens, 1969). As the dry seed undergoes hydration, hydrolysis of 1-octen-3-yl β-primeveroside by β-primeverosidase would occur, potentially explaining the accumulation (Matsui, Takemoto, Koeduka, & Ohnishi, 2018). Increased levels of 1-octen-3-ol were observed in Amelia and Jose (8.1 – 8.8%), but not in Latigo, perhaps suggesting a formation pathway intrinsic to *V. sativa*. Significant proportions of ethanol (>28%) were detected in the chromatograms of all three soaked samples, the extended soaking time (24 h) may have provided suitable quantities of leached soluble carbohydrates to act as substrate for certain wild microbial fermentation.

3.3.3. Germination

Germination significantly increased total volatile relative concentration by 600%, 461% and 303% for Amelia, Jose and Latigo respectively compared to the raw seed controls (Fig. 2a). Proportions of alcohols (16%), furans (9.2%), ketones (4%), and alkenes (1%) were significantly lower than the untreated seed, whilst the levels of sulphides (33%), carboxylic acids (13%), aldehydes (13%), amines (6%), esters (5%) and sulfoxide (0.1%) were significantly higher in vetch seedlings following germination. Within the chromatograms of each sample, dimethyl sulphide was found to be the dominant aroma compound and is understood to form from the conversion of the heat libel *S*-methyl methionine present within the seed endosperm (Bamforth, 2014) during the drying of germinated seed. Germinated vetch was shown to possess decreased levels of off flavor generating compound 1-hexanol, documented as generating undesirable beany and grassy aromas (Roland et al., 2017) within legume seed. Both dimethyl sulphide and 1-hexanol have a relatively low odor threshold which may suggest that they may significantly contribute to the final profile of *V. sativa*.

3.3.4. Microwaving

The microwave cooking of vetch seed samples caused no significant difference to total volatile relative concentration relative to that of raw seed. However, the aroma profiles were found to be substantially different with lower levels of alcohols (9.6%) and alkenes (0.1%); and higher levels of aldehydes (28.6%) compared to the raw seed controls. The compounds 3-methylbutanal, hexanal, and nonanal were detected in microwaved samples and have been previously observed following the microwave treatment of green bean (Rodriguez-Bernaldo De Quiros et al., 2000). Formation of these aldehydes occurs through oxidative degradation of unsaturated fatty acids present within the seed, this occurs in combination with the activity of lipoxygenase and hydroperoxidase lyase enzymes (Roland et al., 2017). The extreme intensity of temperatures produced by dry microwave heating would likely degrade the enzymes responsible for generating many aldehydes, whilst at the same time those temperatures would encourage enhanced autoxidation processes and volatilization, thereby creating a net positive increase in aldehydes within the microwaved seed.

3.3.5. Natto fermentation

Utilization of *B. subtilis* cultures upon vetch to produce natto products yielded significantly higher total relative concentrations compared to the raw vetch seed controls (Fig. 2a). The proportions of pyrazines (20.4%) and carboxylic acids (17.2%) were significantly higher than the raw seed, whilst levels of furans (17.6%), ketones (10%) and alkenes (0.25%) were lower than the untreated vetch seed. Four pyrazine
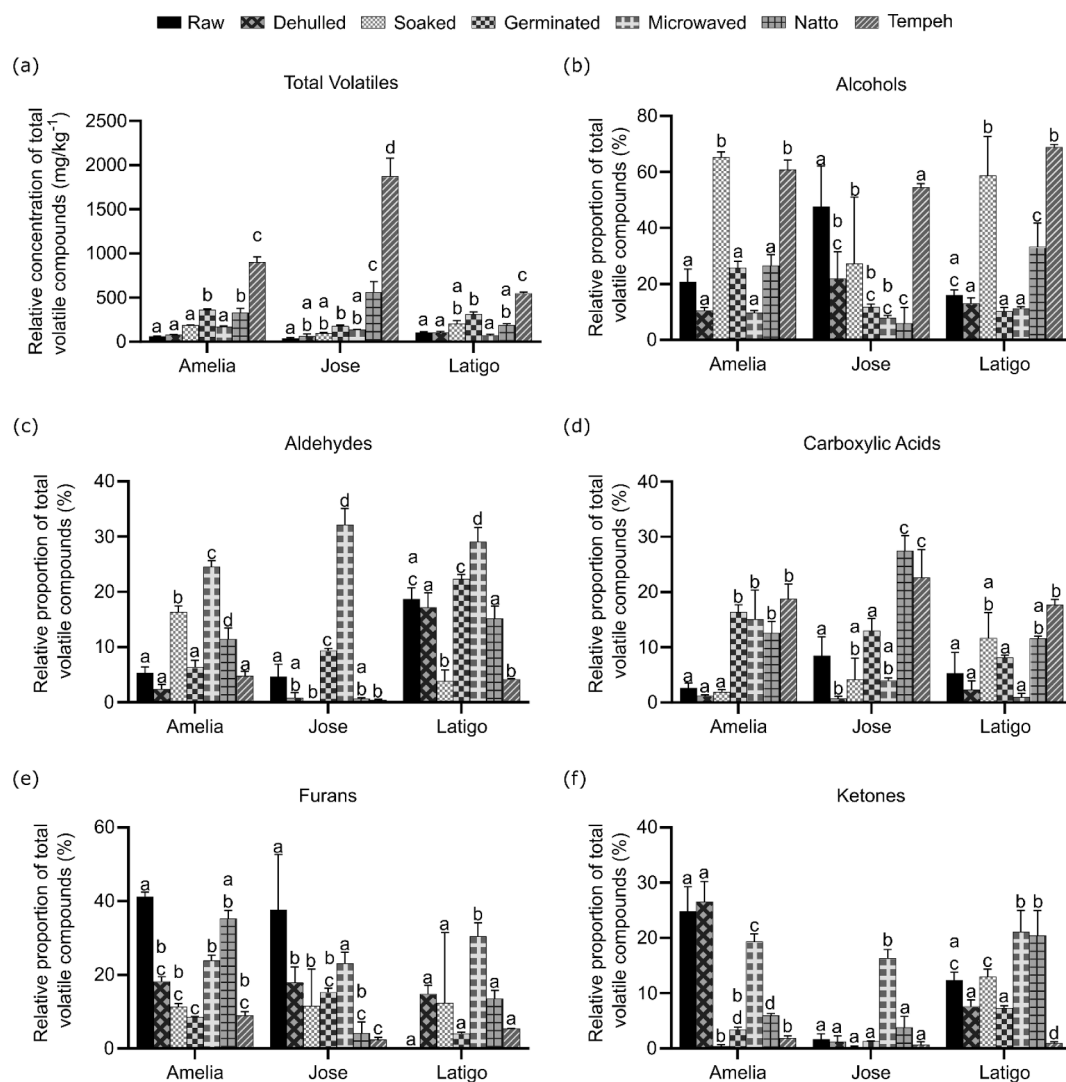
Fig. 2. Effect of processing methods on: (a) the relative concentration of total volatile compounds. (Relative proportions of total volatile compounds were used to demonstrate the functional differences in chemical groups following specific types of processing and thus help to explain the pathways generating production of certain classes of volatiles) (b) the relative proportions of volatile alcohols. (c) the relative proportions of volatile aldehydes; (d) the relative proportions of volatile carboxylic acids; (e) the relative proportions of volatile furans; (f) the relative proportions of volatile ketones present in raw, dehulled, soaked, germinated, microwaved, natto fermented and tempeh fermented *Vicia sativa* (Amelia and Jose) and *Vicia villosa* (Latigo). Results are the average value of three replications. For each variety, mean values bearing different letters are significantly different ($p < 0.05$) as per Tukey's multiple comparisons test.

compounds were detected within the natto fermented vetch including: 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, tetramethylpyrazine and trimethylpyrazine (Table 2). All four pyrazines have been previously identified within natto products produced from soybean (Huang, Liao, Fan, Bai, & Liang, 2012), with 2,5-dimethylpyrazine produced at high concentrations during the final ten hours of fermentation (Liu et al., 2018). The formation of pyrazines is commonly understood to occur because of non-enzymatic mechanisms within both the Maillard reaction and Strecker degradation, primarily involving α-amino acids and reducing sugars (Shu, 1998). *B. natto* is not believed to metabolically synthesize pyrazines directly but instead the products of fermentation including α-acetoacetate, acetoin, free amino acids and ammonia are converted into subsequent pyrazines by non-enzymatic condensation reactions (Rajini et al., 2011). The relative proportions of octen-3-ol and 2-pentyl furan (responsible for characteristic beany aromas) diminished in samples following natto treatment. This infers that the nutty aromas imparted by fermentation-derived pyrazines may be capable of masking the dominant off flavors evident in raw vetch seed, as previously observed in soybean (Kubota & Kobayashi, 1985), thereby achieving food products that are likely to be more palatable to

consumers. Further similarities in aroma profile between fermented vetch and soybean could be observed in the high concentrations of carboxylic acids. These consisted of primarily 3-methylbutanoic acid in natto fermented vetch and the closely related 2 - methylbutanoic acid documented in soybean natto (Liu et al., 2018). Both compounds are characterized by cheesy, rancid, and sweaty aromas (Chung et al., 2005) and are both produced by oxidation of present aldehydes through action of aldehyde dehydrogenases. The aroma profile of fermented vetch natto reflecting so closely that produced by soybean indicates that fermented vetch has the potential to be deemed acceptable by consumers in comparable ways to that of conventional soybean natto products.

3.3.6. Tempeh fermentation

Compared to the samples derived from natto fermentation, tempeh fermented vetch samples exhibited over three-fold higher total relative concentrations (Fig. 2a). This could be explained by the longer incubation time (72 h) required by tempeh to achieve complete fermentation of vetch, compared with only 20 h for natto fermentation, reflecting the relative metabolism of *R. oligosporus* compared to *B. subtilis*. Tempeh fermentation yielded substantial compositional differences relative to

raw samples; the levels of alcohols (61.4%) and carboxylic acids (19.7%) were significantly greater, whilst aldehydes (3.1%), alkenes (2.3%), furans (5.6%) and ketones (1.2%) were notably lower. Of the volatile compounds detected in samples of vetch tempeh, only 3-methyl butanol (described as producing a rancid odor) was found to have been previously described in literature relating to characteristic aromas of traditional soybean tempeh (Jeleń et al., 2013). Formation of 3-methyl butanol following the reduction of 3-methyl-1-butanal, a reaction found to be significantly enhanced in soybean tempeh once frying had occurred (Jeleń et al., 2013). In a broader study investigating the release of aroma volatiles from tempeh fermented malt extract agar, barley and soybean (Mei Feng et al., 2007), a larger number of aroma volatiles were

described and a subsequent larger proportion of these were detected in vetch tempeh. The commonly identified compounds detected within both the malt extract agar and soybean tempeh included that of ethanol, 3-methyl-1-butanol and dimethyl sulphide, whilst specifically detected in the malt extract agar were significant levels of phenylethyl alcohol, ethyl acetate and 2,3-butanediol. The formation of these particular alcohols in such quantities is a strong indicator that an enzymatic lipoxygenase pathway is present in vetch, as the pathway is required for alcohol production during soybean fermentation (Jeleń et al., 2013). The comparable aroma profiles of fermented vetch to that of soybean, malt extract and barley suggest that fermented vetch has the potential to be deemed palatable/acceptable to consumers, and as a result swiftly
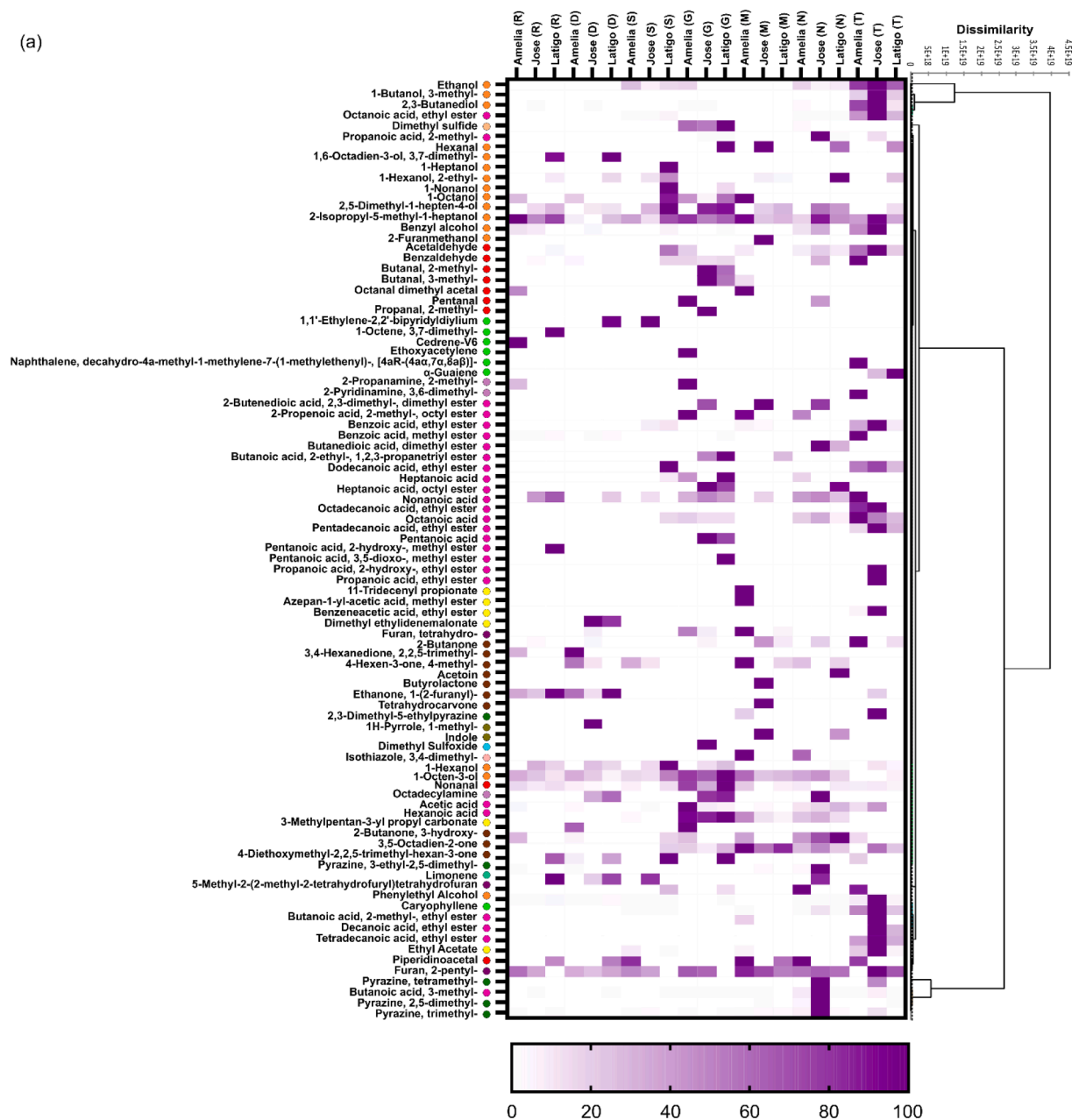
Fig. 3. (a) Hierarchical cluster analysis of *V. sativa* varieties Amelia and Jose and *V. villosa* variety Latigo subject to typical seed processing protocols (R: raw, D: dehulled, S: soaked, G: germinated, M: microwaved, N: natto fermentation, T: tempeh fermentation) and identified volatile compounds. Colours in the heatmap indicate the normalised relative concentrations of volatiles; purple for higher levels, white for lower levels. Colour circles after the name of the compound describes the compound class that each particular compound belongs to: red, aldehyde; brown, ketone; orange, alcohol; yellow, ester; indigo, furan; pink, carboxylic acids; light blue, alkenes; dark green, pyrazines; cyan, sulfoxides; dark blue, amines; cream, sulphides; grey, pyrroles; light green, terpenes; hot pink, thiazoles. (b) Heatmap of the correlation matrix of the volatile compounds with the main clusters situated along the diagonal line (groups a-f). Positive correlations between volatile compounds are shown in blue; negative correlations in red; absence of correlation in white.
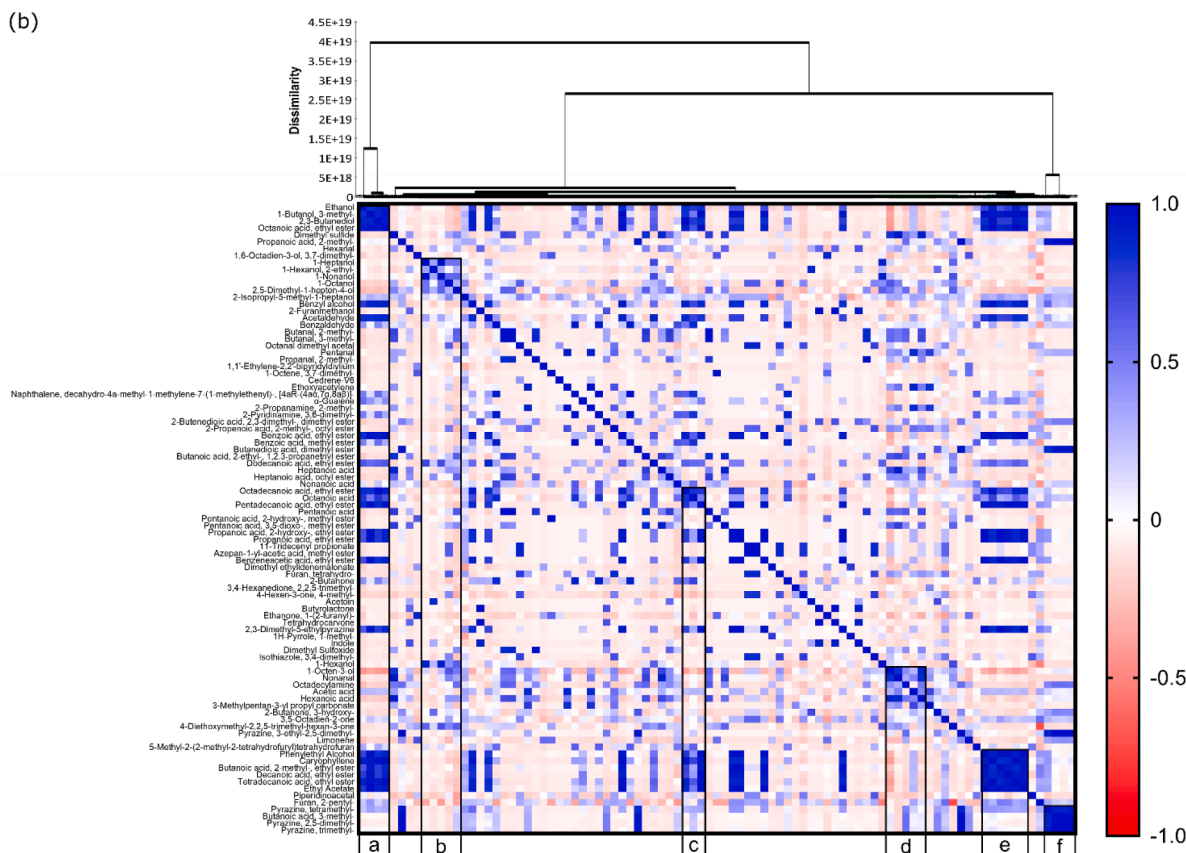
(b)

Fig. 3. (*continued*).

accepted as a future food ingredient.

3.3.7. Aroma volatile grouped behaviors

Multivariate analysis (MVA) was performed (Fig. 3b) to investigate the clustering of aroma volatiles and explore their potentially related biochemical formations. MVA revealed a total of six significant clustering events. The correlation clusters 'a', 'c' and 'e' contained individual groups of compounds collectively present at high concentrations following tempeh fermentation of vetch, all of which shared high levels of intercluster correlation. The individual clusters were primarily differentiated by compound class and the routes by which they were metabolized. Cluster 'a' was primarily composed of the alcohols: ethanol and 3-methyl-1-butanol generated by the reduction of acetaldehyde during aerobic fermentation and 2,3-butanediol formed by the reduction of acetoin via comparable biological mechanisms. Correlation cluster 'c' (octadecanoic acids, ethyl ester, octanoic acid and pentadecanoic acid, ethyl ester) and 'e' (butanoic acid, 2-methyl-, ethyl ester, decanoic acid, ethyl ester and tetradecanoic acid, ethyl ester) comprised primarily of carboxylic acids, all of which were derived from the oxidation of primary alcohol volatiles. The distinct separation of clustering events observed can therefore be classified as those metabolism products formed during the early (cluster 'a') and late (cluster 'c' and 'e') stages of vetch tempeh fermentation. Such distinct separation of clusters 'c' and 'e' despite similar aroma volatile chemical properties could be explained by the presence of the alkene caryophyllene and phenylethyl alcohol within cluster 'e'.

Association cluster 'f' contained primarily highly associated pyrazine volatile compounds which included tetramethyl pyrazine, 2,5-dimethyl pyrazine and trimethyl pyrazine. The association of these compounds within cluster 'f' can be explained by their collective occurrence at high concentrations within all fermented natto samples. This is in line with previous observations that the formation of pyrazines is likely due to

non-enzymatic condensation reactions of the early fermentation products; α-acetoacetate, acetoin, free amino acids and ammonia.

The remaining clusters, 'b' and 'd' had less definitive positive correlations than that of the previously mentioned clusters. Grouping for cluster 'b' was entirely between alcohols including 1-heptanol, 1-hexanol, 2-ethyl,1-nonanol, 1-octanol and 2,5-dimethyl-1-hepten-4-ol. These compounds were particularly abundant in soaked samples and were likely produced by the wild microbial fermentation of leached soluble carbohydrates as previously described. The association of compounds within cluster 'd' was the least obvious of those highlighted by multivariate analysis, this was primarily due to the representation of a diverse range of compound classes including alcohols, aldehydes, amines, carboxylic acids and esters. Upon further analysis the compounds were all found at high concentrations within germinated vetch samples and the wide variety of volatile metabolites are typical of the vast array of metabolic processes that occur during initial germination.

Whilst not the focus of the study, it should be noted that individual aroma compounds have varying odor thresholds. Odor thresholds indicate the concentration at which the compound can be perceived, which is typically measured in an aqueous or aqueous-ethanol systems. Some compounds (e.g. sulphur containing volatiles) will have odor thresholds over 100 times lower than other compounds, but due to the structural complexity of the vetch material and the synergistic effects that occur when they are perceived, these simplified interpretations of the contributions to overall aroma profiles do not always represent reality in the final product, however they do give an indication to the relative importance of single compounds to perception and should be taken into account in future studies.

4. Conclusion

As breeding programs rapidly accelerate progress towards a zero

GBCA vetch, there is an imperative to understand how vetch composition responds to common food processing approaches. A true understanding of the link between vetch genetics, vetch chemistry and processing upon flavor will be crucial in the targeted design of vetch as a mainstream protein source for consumers around the world.

Through this first utilization of fingerprint SPME analysis of vetch seeds and products, the main volatile constituents of *V. sativa* were found to be furans, alcohols, ketones, carboxylic acids, aldehydes and alkenes. 2-Pentyl furan, benzyl alcohol, benzaldehyde, 1-octen-3-ol, and 1-hexanol were found to be the most abundant volatiles present in *V. sativa* raw seed, but processing caused a major alteration to the overall aroma profiles of samples. Dehulling resulted in ester production, namely 3-methylpentan-3-yl propyl carbonate and dimethyl ethylidenemalonate, which formed from the partial oxidation of alcohols and carboxylic acids in the raw seed. Soaking vetch caused a substantial increase in alcohols, specifically ethanol and 1-octen-3-ol, as soluble carbohydrates leached into the soaking liquid and encouraged subsequent fermentation by wild microorganisms. Considerably increased levels of those alcohols, as well as 3-methyl-1-butanol, phenylethyl alcohol and 2,3-butanediol were observed following tempeh fermentation. Alternatively, natto fermentation generated notable increases in pyrazines as the initial products of biological fermentation were altered by non-enzymatic condensation reactions downstream. Microwave cooking generated significant quantities of aldehydes, primarily 3-methylbutanal, hexanal, and nonanal formed by oxidative degradation of unsaturated fatty acids as well as their degradation by lipoxygenase and hydroperoxidase lyase enzymes; these processes were likely accelerated at the increased temperatures. Germination of vetch seed caused increased levels of sulphides, specifically dimethyl sulphide originating from the mechanistic conversion of the heat libel *S*-methyl methionine within the seed endosperm during the drying process before analysis.

Overall, post-harvest processing methods notably altered the composition of the samples, with specifically germination, natto and tempeh fermentation significantly increasing the total volatile relative concentration compared to the raw seed. An increased understanding of the volatile compounds within vetch will improve opportunities for its development as a safe and sustainable protein source.

CRediT authorship contribution statement

Samuel Riley: Methodology, Investigation, Formal analysis, Writing – original draft. **Aneesh Lale:** Resources, Writing – review & editing. **Vy Nguyen:** Resources, Writing – review & editing. **Hangwei Xi:** Methodology, Writing – review & editing. **Kerry Wilkinson:** Supervision, Methodology, Writing – review & editing. **Iain R. Searle:** Conceptualization, Supervision, Writing – review & editing. **Ian Fisk:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133569.

References

Akkad, R., Kharraz, E., Han, J., House, J. D., & Curtis, J. M. (2019). Characterisation of the volatile flavour compounds in low and high tannin faba beans (Vicia faba var. minor) grown in Alberta, Canada. *Food Research International, 120*(December 2018), 285–294. 10.1016/j.foodres.2019.02.044.

Badenhop, A. F., & Wilkens, W. F. (1969). The formation of 1-octen-3-ol in soybeans during soaking. *Journal of the American Oil Chemists Society, 46*(3), 179–182. https://doi.org/10.1007/BF02635729

Bamforth, C. W. (2014). Dimethyl sulfide - Significance, origins, and control. *Journal of the American Society of Brewing Chemists, 72*(3), 165–168. https://doi.org/10.1094/ASBCJ-2014-0610-01

Chung, H. Y., Fung, P. K., & Kim, J. S. (2005). Aroma impact components in commercial plain sufu. *Journal of Agricultural and Food Chemistry, 53*(5), 1684–1691. https://doi.org/10.1021/jf048617d

Depristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., Philippakis, A. A., Del Angel, G., Rivas, M. A., Hanna, M., McKenna, A., Fennell, T. J., Kernytsky, A. M., Sivachenko, A. Y., Cibulskis, K., Gabriel, S. B., Altshuler, D., & Daly, M. J. (2011). A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics, 43*(5), 491–501. https://doi.org/10.1038/ng.806

Eicher, C. K. (2006). Staff Paper. *Agricultural Economics, August*, 3–4.

Enopala, J. T., González, F. J., & De La Barrera, E. (2012). Physiological responses of the green manure, vicia sativa, to drought. *Botanical Sciences, 90*(3), 305–311. https://doi.org/10.17129/botsci.392

Gomes, J., Jadrić, S., Winterhalter, M., & Brkić, S. (1982). Alcohol dehydrogenase isoenzymes in chickpea cotyledons. *Phytochemistry, 21*(6), 1219–1224. https://doi.org/10.1016/0031-9422(82)80114-3

Hollingsworth, R. G. (2005). Limonene, a citrus extract, for control of mealybugs and scale insects. *Journal of Economic Entomology, 98*(3), 772–779. https://doi.org/10.1603/0022-0493-98.3.772

Hudaib, T., Hayes, W., Brown, S., & Eady, P. E. (2010). Effect of seed moisture content and D-limonene on oviposition decisions of the seed beetle Callosobruchus maculatus. *Entomologia Experimentalis et Applicata, 137*(2), 120–125. https://doi.org/10.1111/j.1570-7458.2010.01044.x

Jansen, L., Parizot, B., & Beeckman, T. (2013). Inducible system for lateral roots in arabidopsis thaliana and maize. In *Methods in Molecular Biology* (Vol. 959). 10.1007/978-1-62703-221-6_9.

Jeleń, H., Majcher, M., Ginja, A., & Kuligowski, M. (2013). Determination of compounds responsible for tempeh aroma. *Food Chemistry, 141*(1), 459–465. https://doi.org/10.1016/j.foodchem.2013.03.047

Kettlitz, B., Scholz, G., Theurillat, V., Cselovszky, J., Buck, N. R., O' Hagan, S., Mavromichali, E., Ahrens, K., Kraehenbuehl, K., Scozzi, G., Weck, M., Vinci, C., Sobieraj, M., & Stadler, R. H. (2019). Furan and Methylfurans in Foods: An Update on Occurrence, Mitigation, and Risk Assessment. *Comprehensive Reviews in Food Science and Food Safety, 18*(3), 738–752. https://doi.org/10.1111/1541-4337.12433

Khrisanapant, P., Kebede, B., & Leong, S. Y. (2019). A Comprehensive Characterisation of Volatile and. *Foods, 8*(651), 1–19.

Kouris-Blazos, A., & Belski, R. (2016). Health benefits of legumes and pulses with a focus on Australian sweet lupins. *Asia Pacific Journal of Clinical Nutrition, 25*(1), 1–17. https://doi.org/10.6133/apjcn.2016.25.1.23

Kubota, K., & Kobayashi, A. (1985). Comparison of Compositions of Odor Components of Natto and Cooked Soybeans. *Agricultural and Biological Chemistry, 49*(2), 311–317. https://doi.org/10.1271/bbb1961.49.311

Lee, T. H., Guo, H., Wang, X., Kim, C., & Paterson, A. H. (2014). SNPhylo: A pipeline to construct a phylogenetic tree from huge SNP data. *BMC Genomics, 15*(1), 1–6. https://doi.org/10.1186/1471-2164-15-162

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics, 25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Liu, Y., Song, H. L., & Luo, H. (2018). Correlation between the key aroma compounds and gDNA copies of Bacillus during fermentation and maturation of natto. *Food Research International, 112*(11), 175–183. https://doi.org/10.1016/j.foodres.2018.06.033

Mao, Z., Fu, H., Nan, Z., & Wan, C. (2015). Fatty acid, amino acid, and mineral composition of four common vetch seeds on Qinghai-Tibetan plateau. *Food Chemistry, 171*, 13–18. https://doi.org/10.1016/j.foodchem.2014.08.090

Matsui, K., Takemoto, H., Koeduka, T., & Ohnishi, T. (2018). 1-Octen-3-ol Is Formed from Its Glycoside during Processing of Soybean [Glycine max (L.) Merr.] Seeds. *Journal of Agricultural and Food Chemistry, 66*(28), 7409–7416. https://doi.org/10.1021/acs.jafc.8b01950

Mei Feng, X., Ostenfeld Larsen, T., & Schnürer, J. (2007). Production of volatile compounds by Rhizopus oligosporus during soybean and barley tempeh fermentation. *International Journal of Food Microbiology, 113*(2), 133–141. https://doi.org/10.1016/j.ijfoodmicro.2006.06.025

Nguyen, V., Riley, S., Nagel, S., Fisk, I., & Searle, I. R. (2020). Common Vetch: A Drought Tolerant, High Protein Neglected Leguminous Crop With Potential as a Sustainable Food Source. *Frontiers in Plant Science, 11*(June), 1–7. https://doi.org/10.3389/fpls.2020.00818

Noordraven, L. E. C., Buvé, C., Chen, C., Hendrickx, M. E., & Van Loey, A. M. (2021). Impact of Processing and Storage Conditions on the Volatile Profile of Whole Chickpeas (Cicer arietinum L.). ACS. *Food Science & Technology, 1*(6), 1095–1108. https://doi.org/10.1021/acsfoodscitech.1c00108

Otera, J., & Nishikido, J. (2009). *Esterification Methods, Reactions, and Applications Escrito por Junzo Otera, Joji Nishikido*.

Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter, 15*(1), 8–15. https://doi.org/10.1007/BF02772108

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., De Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics, 81*(3), 559–575. https://doi.org/10.1086/519795

Rajini, K. S., Aparna, P., Sasikala, C., & Ramana, C. V. (2011). Microbial metabolism of pyrazines. *Critical Reviews in Microbiology, 37*(2), 99–112. https://doi.org/10.3109/1040841X.2010.512267

Roberts, L. (2011). Population is growing —fast. *Science, 333*(6042), 540–543.

Rodriguez-Bernaldo De Quiros, A. I., López-Hernández, J., González-Castro, M. J., De La Cruz-García, C., & Simal-Lozano, J. (2000). Comparison of volatile components in raw and cooked green beans by GC-MS using dynamic headspace sampling and microwave desorption. *European Food Research and Technology, 210*(3), 226–230. https://doi.org/10.1007/PL00005517

Roland, W. S. U., Pouvreau, L., Curran, J., Van De Velde, F., & De Kok, P. M. T. (2017). Flavor aspects of pulse ingredients. *Cereal Chemistry, 94*(1), 58–65. https://doi.org/10.1094/CCHEM-06-16-0161-FI

Schindler, S., Wittig, M., Zelena, K., Krings, U., Bez, J., Eisner, P., & Berger, R. G. (2011). Lactic fermentation to improve the aroma of protein extracts of sweet lupin (Lupinus angustifolius). *Food Chemistry, 128*(2), 330–337. https://doi.org/10.1016/j.foodchem.2011.03.024

Shu, C. K. (1998). Pyrazine Formation from Amino Acids and Reducing Sugars, a Pathway Other than Strecker Degradation. *Journal of Agricultural and Food Chemistry, 46*(4), 1515–1517. https://doi.org/10.1021/jf970999i

The Eat-Lancet Commission. (2019). Healthy Diets From Planet; Food Planet Health. *The Lancet, 32*.

Valentine, S. C., & Bartsch, B. D. (1996). Production and composition of milk by dairy cows fed common vetch or lupin grain as protein supplements to a silage and pasture-based diet in early lactation. *Australian Journal of Experimental Agriculture, 36*(6), 633–636. https://doi.org/10.1071/EA9960633

Wilkens, W. F., & Lin, F. M. (1970). Gas Chromatographic and Mass Spectral Analyses of Soybean Milk Volatiles. *Journal of Agricultural and Food Chemistry, 1*, 333–336. https://doi.org/10.1021/jf60169a003

X. Huang, L.Y. Liao, L. Fan, L. Bai, Y.B. Liang, L. W. J. (2012). Analysis of volatile components from natto by solid-phase microextraction. *Science and Technology of Food Industry, 33*(2012), 58–61.

Xi, H., Nguyen, V., Ward, C., Liu, Z., & Searle, I. R. (2021). Chromosome-level assembly of the common vetch reference genome (Vicia sativa). *BioRxiv*, 2021.10.11.464017. 10.1101/2021.10.11.464017.