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Aroma and metabolite profiling in duckweeds: Exploring species and ecotypic variation to enable wider adoption as a food crop

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ABSTRACT

Duckweeds (water lentils) are a nutritious human food source, with *Wolffia* species consumed traditionally in Eastern Asia. Duckweed contain up to 45 % protein by dry weight, high macronutrients, minerals and carotenoids. However, duckweed are not cultivated at scale and there are circa 35 other species to consider for food potential in other global regions. Here, we measured the suitability of four *Lemna* species and *Spirodela polyrhiza* for nutritional assessment, by scaling up growth of 25 ecotypes from the United Kingdom in a glasshouse. Here we showed intra- and inter-species variation of aromatic and metabolic profiles, together with biomass obtained from production. The dominant volatile organic compounds (VOCs) in duckweed are hexanal, 1-penten-3-one, 1-penten-3-ol, *cis*-2-pentanol and pentadecanal, with variations in amounts of 22 other compounds between species. In comparison with other leafy herbs, duckweed aroma profiles were most similar to spinach and dandelion with high 'green' and 'fresh' aroma compounds. *Spirodela polyrhiza* contained high flavonoids including apigenin and luteolin, offering potential benefits for health. Our results demonstrate that *Lemna* and *Spirodela* species have suitable flavonoid and amino acid profiles for nutrition. VOCs found here had positive aroma descriptors and can be used as biomarkers of freshness during storage of duckweed foodstuffs.

1. Introduction

Duckweeds, also known as water lentils, offer exceptionally rapid vegetative growth and high global availability, providing a sustainable alternative to both animal and plant protein such as soybean [1]. Duckweeds are additionally used in circular economy projects to produce animal and fish feeds [2,3] and are proposed for human food production due to their versatility for growth in outdoor ponds and vertical farms [4]. Small space requirements and fast growth make some species promising for space horticulture [5,6]. Duckweeds contain up to 30–45 % total dry weight protein, all nine essential amino acids, are high in potassium and iron [7–9] and contain high levels of carotenoids, especially lutein and zeaxanthin [10–12]. Furthermore, the starch content can exceed 70 %, making duckweed a potential carbohydrate source for food and biofuel [13].

Several rootless species have traditionally been used in Asian dishes known as "Khai-nam" (*Wolffia arrhiza* and *Wolffia globosa*), and are now commercially cultivated as the supergreen "Mankai" [14,15]. *Wolffia* species contain bioavailable amino acids above the world health organisation (WHO) recommended levels [9,16]. "Mankai" contains 200 polyphenol compounds from flavonoid and phenolic acid groups, with associated potential anti-cancer, anti-inflammation and anti-microbial properties [17,18]. However, with 36 species of duckweed to choose from globally, not all species have been equally considered as food crops. Additionally, variation in growth, protein, starch content, and available minerals among different species and ecotypes of duckweed

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have been reported [8,19,20] and furthermore will be dependent on environment and available nutrients.

More recently, other genera of duckweeds have also been evaluated for food applications, especially in regions where *Wolffia* is less prevalent (e.g. Europe). When combined in a chicken feed, dried *Lemna* and *Spirodela* species of duckweeds exhibited high amino acid digestibility [21]. *Lemna minor* protein powder now has GRAS status in the USA and is undergoing regulation in the EU as a novel food [22]. Moreover, extraction of Ribulose bisphosphate carboxylase/oxygenase (Rubisco) from mixed *Lemna minor* and *Lemna gibba* species is considered safe in Europe [23], and has been incorporated as an animal protein analogue [24].

Rooted duckweed species from the genera *Lemna* and *Spirodela* have uses in the treatment of allergies, inflammation, and tumours [25–27], likely these properties can be attributed to antioxidants, including flavonoids in *Lemna minor*, *Spirodela polyrhiza* and *Landoltia punctata* [28–31]. *Lemna minor* pills are manufactured as an herbal remedy available in the USA [32] and *Lemna minor* extracts exhibit antibacterial and antifungal activity against food spoilage microorganisms [33–35] and possess pesticidal properties against weeds [36,37]. Despite this, volatile and metabolite profiles have not been characterised largely between species and bioactivity of specific compounds have not been related to these functions.

Aroma perception is based on the unique combination of aroma compounds and their respective odour thresholds. Scents are detected by olfactory receptors both orthonasally during sniffing and retronasally during chewing, and this is key for human decision-making about edibility and safety of foods. The excess production of certain volatile organic compounds (VOCs), which are associated with undesirable smells, can limit the shelf-life of food. This is evident in leafy salads such as spinach, where the accumulation of off-notes are a limiting factor in post-harvest storage [38,39]. Enhancing compounds within duckweed aroma profiles attributed with 'pleasant' and reducing those associated with 'malodourous' could therefore be used for selecting species and ecotypes with enhanced appeal. Moreover, plant production of VOCs can act as defensive compounds against herbivores and insects, which may enhance crop resilience and foodstuff storage potential [40]. Therefore, understanding intra- and inter-species variation in VOCs, and their complex metabolic pathways could be used in synthetic engineering of future crop varieties [41].

Despite the promise of duckweeds as a new food source, resistance to duckweed acceptability in Western Europe was identified in some consumers due to association with unclean water [42]. Moreover, the drive for sustainable and resilient food systems includes using novel plant ecotypes which are well adapted to a local growing environment, stimulating regional economy and giving shorter distances for transport [43]. To address these shortcomings, we compared organoleptic value and nutritional properties among UK-derived duckweeds composed of *Lemna* species and *Spirodela.* These ecotypes previously showed variation in growth, adaptation to high light and carotenoid contents [12]. Aroma (VOC) profiles are discussed in the contexts of human acceptability for consumption and shelf-life. The aim is to recommend duckweed candidates for sustainable food development within the UK and Europe.

2. Materials and methods

2.1. Selection of duckweed ecotypes and herbs

Twenty-five duckweed ecotypes within four *Lemna* species and one *Spirodela* species were selected: *L. minor*, *L. japonica*, *L. minuta*, and *L. turionifera* and *S. polyrhiza*. Ecotypes were chosen from a UK duckweed collection consisting of >100 ecotypes which were collected between 17/05/20 and 15/07/22. Species were identified using next generation sequencing and the selected ecotypes were previously grown on a small-scale in a controlled light environment, showing varied growth rates and tolerance to light [12], The ecotypes used are detailed in Fig. S1 and summarized in Tables S1 and S2. Other leafy green vegetables were sourced as seeds including spinach (Mr Fothergills, UK), and aromatic herbs including coriander (Mr Fothergills, UK) and red sweet basil (D.T Brown, UK) from a local garden centre and used as comparative controls for aroma profiling described below.

2.2. Glasshouse system for growth of duckweed ecotypes and herbs

Large-scale duckweed production was carried out for six months during winter 2021 to spring 2022 at Sutton Bonington campus, University of Nottingham, UK. Four batches of 25 duckweed ecotypes were grown simultaneously in quadruplicate (see Fig. 1), these formed randomly positioned replicates, around a glasshouse offering 7 m^2 total growing space. Duckweed ecotypes were set up using three healthy three-frond colonies within black seed trays (32.5 × 22.5 × 5 cm) containing 1 dm^3 Nutrient (N) medium covered with Plastic propagator lids Apet (H. Smith plastics, UK). Each tray was harvested every two months, except in the slower growing ecotypes, which were harvested when trays had 95 % duckweed surface coverage. After each harvest, three colonies were used to restart growth. A maximum of three harvests over six months were completed from each tray, with an experimental end point in April 2022 (Fig. 1). Commercial seeds of spinach, coriander and red basil were grown in the same conditions in seed trays of Levington M3 soil, for subsequent aroma profiling.

2.2.1. Nutrition and growing environment

Duckweeds were grown on a large scale and non-aseptically, representing potential commercial growing conditions. N-medium is an optimum duckweed growing media described in Ref. [44], consisting of KH_2PO_4 (0.15 mM), $Ca(NO_3)_2$ (1 mM), KNO_3, (8 mM), $MgSO_4$ (1 mM), H_3BO_3 (5 μM), $MnCl_2$ (13 μM), Na_2MoO_4 (0.4 μM), and FeEDTA (25 μM) with traces of Si, Cu and Zn. N-medium was made with reverse osmosis water and sterilized at 121 °C and replaced weekly in each tray to maximize nutrient dosage. Duckweeds were washed with reverse osmosis water in sieves and returned to trays containing fresh media weekly. At timepoints in spring, media was topped up weekly with 1 dm^3 reverse osmosis water when evaporation was visible.

Duckweeds and herbs were grown in temperatures set at 23 °C and 21 °C day and night and monitored using a datalogger TGU-4500 (Gemini, UK) with this data presented in Fig. S2. Duckweeds and herbs were grown in natural day light supplemented with high pressure sodium bulbs, supplying a total maximum light intensity of 180 μmol photons m^{-2} s^{-1}. An extended photoperiod of 16 h was provided, with supplementary lighting between 7 a.m. and 23 p.m. Light intensity and light quality were measured above each replicate tray using a light meter LI-250A (LI-COR, Biosciences, NE, USA) and a handheld spectrometer LI-180 (LI-COR, Biosciences, NE, USA) (Fig. 1). All light measurements are presented in Tables S3A and 3B.

2.2.2. Measurements of duckweed health

Photographs were taken after four weeks of growth (Fig. 1) with a Canon 650D camera (Canon Inc., Tokyo, Japan) 40 cm above each tray with the whole tray in the field of view. Average greenness value and fraction cover of duckweed per tray were obtained from images and used to measure duckweed health and growth. Each parameter was derived using Fiji image processing software using five random rectangles [45] per image as described in Ref. [12]. Average greenness was obtained by extracting red-green-blue (RGB) values using ten regions within each rectangle. Growth as percentage coverage of green biomass was calculated relative to background area in each rectangle from photographs.

As a proxy for duckweed health and growth, reflectance data was collected using an ASD Fieldspectrometer (ASD Field Spec 4, Malvern Panalytical, UK) after four weeks growth (Fig. 1). Reflectance of duckweed biomass was measured with the sensor's optic fibre at 20 cm above each tray, at 1 nm increments between 350 nm and 2500 nm. Three full

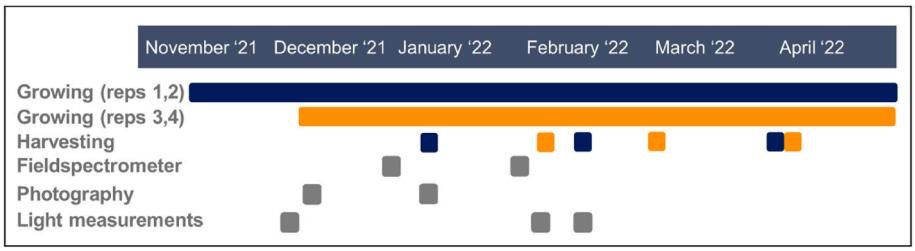

Fig. 1. Gannt chart indicating setup, harvest and end-point dates of duckweed glasshouse experiment. Growth and harvesting periods of each duckweed ecotype replicates 1 and 2 are indicated in blue and replicates 3 and 4 in orange. Photographs, fieldspectrometer and light measurement timepoints are indicated in grey and were collected for all replicates. Photograph and fieldspectrometer measurements were used for comparing duckweed health and growth during the experiment for comparison with subsequent harvesting data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

range spectral measurements were made per tray. To estimate crop biomass and Nitrogen status, vegetation indices were calculated from reflectance data using spectral ratios of raw reflectance in the green and near infra-red regions. Nitrogen status was estimated using Other vegetation index (OVI) [46], and estimates for plant greenness and biomass used the following vegetation indices: Normalised difference vegetation index (NDVI) [47], Green index (GI) [48] and Green model (GM) [49]. All vegetation indices were calculated from reflectance where R corresponds to the wavelength of the measured reflectance in the following formulas:

$$OVI = \frac{R_{760}}{R_{730}} \left[46\right] \quad \text{(i)}$$

$$NDVI = \frac{R_{800} - R_{680}}{R_{800} + R_{680}} \left[47\right] \quad \text{(ii)}$$

$$GI = \frac{R_{554}}{R_{667}} \left[48\right] \quad \text{(iii)}$$

$$GM = \frac{R_{750}}{R_{550}} - 1 \left[49\right] \quad \text{(iv)}$$

2.3. Harvesting duckweed biomass

During each harvest, whole trays of duckweed ecotypes were washed with reverse osmosis water in sieves. Fresh biomass was then air-dried in the glasshouse for 15 min. Duckweed biomass was weighed from each tray to obtain fresh biomass per harvest. Biomass was then frozen in liquid nitrogen and stored at −80 °C until further aroma and metabolite processing.

2.4. Preparation of plant tissue

For aroma profiling, basil, coriander and spinach samples were collected from glasshouse-grown tissue and dandelion leaves were harvested from wild plants growing in Sutton Bonington, UK woodland area ($n = 4$ per herb). Duckweeds and herbs were freeze-dried for two days and re-weighed. Freeze-dried biomass were then ball-milled to a fine powder using a RETSCH PM400 ball mill (Haan, Germany). Fine freeze-dried duckweed and herb powders were then stored at −80 °C until aroma and metabolite analysis.

2.5. Aroma profiling

2.5.1. Preparation of samples for aroma profiling using SPME-GCMS

Duckweed powder (0.5 g) or dried herb samples (0.5 g) were weighed into Solid phase microextraction (SPME) amber vials. MilliQ (Merck Millipore) water (4 cm^3) and internal standard (0.001 cm^3 0.001 % 3-Heptanone in methanol (MeOH) v/v) was added to the dried samples. Samples were prepared over a two-week period with a random sampling design to process independent replicates of each ecotype from four locations within the glasshouse (25 duckweed ecotypes, $n = 4$, other herbs $n = 4$). Samples were then analysed using Solid phase microextraction Gas chromatography/mass spectrometry (SPME-GCMS).

2.5.2. SPME-GCMS to determine aroma profiles of duckweed

An untargeted volatilome approach was used to discover and semi-quantify volatile compounds. Sample volatiles were extracted from vial headspace for 30 min at 50 °C using 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, Sigma Aldrich, UK) followed by desorption for 1 min at 250 °C in spitless mode using a TriPlus robotic sample-handling (RSH) Autosampler. Analysis was conducted on a Thermo Scientific™ ISQ™ single quadruple mass spectrometer with a TRACE™ 1300 gas chromatography (GC) system. Separation was performed on a 30 m Zebron (ZB) wax column with inner diameter of 0.25 mm and 1 μm film thickness (Phenomenex Inc., Macclesfield, UK) using 18 PSI constant Helium pressure and mass separation (MS) full scan mode resolving mass to charge ratios (m/z) between 35 and 300.

2.5.3. Human aroma perception using GC-olfactory (GC-O) analysis

A small panel of five participants were selected for Gas chromatography -olfactory- mass spectrometry (GC-O-MS) for preliminary human perception of duckweed aroma. *Lemna japonica* KS18 freeze-dried powder was chosen for its high quantity. Sample volatiles were extracted using the same method in 2.5.1. and 2.5.2. but using a GC machine (TRACE 1300 GC, USA, and ISQ™ series mass spectrometer MS) customized with an olfactometry detector outlet [50]. Participants were asked to record times, descriptions and intensities of aromas. Those compounds most frequently reported at similar times during extraction contributed to aroma perception of duckweed.

2.5.4. Data processing of aroma profiles

Peak detection and integration was performed from raw data using TraceFinder 5.1 (Thermo Fisher Scientific) with deconvolution plugin 1.2. Spectral reference libraries (NIST/EPA/NIH Mass spectral library 2.0, National institute of Science and technology, Gaithersburg, MD) were used to identify compounds based on retention index and polar index. Retention time alignment was performed on all duckweed and herb samples using a threshold index of 100,000. A standard panel of Alkanes (C6–C20) (Sigma Aldrich, UK) were used to obtain linear retention index (LRI) for compound identification. Concentration of volatile compounds (μg/kg) were expressed relative to the ratio of compound peak area to the internal standard peak area.

For aroma analysis (and metabolite analysis, see 2.6. below), five species groups were formed from four replicates of 25 ecotypes: individual ecotypes within each species group were $n = 10$ *L. japonica*, $n = 5$ *L. minor*, $n = 5$ *L. minuta*, $n = 2$ *L. turionifera*, $n = 3$ *S. polyrhiza*. The total replicates per species were: *L. japonica* = 40, *L. minor* = 20, *L. minuta* =

20, *L. turionifera* = 8 and *S. polyrhiza n* = 12.

For each herb, the total replicates were $n = 4$. A Games-Howell post-hoc test was used to determine differences in amounts of VOCs between duckweed and herb pairs. Kruskal-Wallis and Wilcoxon paired post-hoc test with Benjamini-Hochberg correction were used to find inter-species differences for individual compounds. P = <0.05 was set for the significance boundary in each case.

For GC-O analysis, nasal impact frequencies (NIFs) were used to identify compounds with >50 % participant detection. These compounds contributed the most to aroma perception and are plotted as an aromagram. To determine the impact of each aroma compound relative to its concentration, odour activity values (OAVs) were calculated using odour thresholds from Ref. [51]. OAV is determined from the ratio of odour threshold and concentration in duckweed (μg/kg) whereby the lowest odour threshold and highest concentration give the greatest aroma contribution.

$$OAV = \frac{concentration}{odour\ threshold} \quad \text{(v)}$$

Odour descriptors for compounds were obtained from The Good Scents Company (thegoodscentscompany.com [52]) to associate compounds with pleasant or unpleasant aromas.

2.6. Metabolite analysis

2.6.1. Metabolite analysis via LC-MS/MS and HPLC-PDA

Metabolite analysis for soluble sugar, starch, free amino acids and secondary metabolites including flavonoid phenolic compounds was conducted with Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) as described in Ref. [53]. Secondary metabolites were additionally measured by High-performance liquid chromatography-Photo diode array (HPLC-PDA). The following adjustments were made: change of the LC-MS/MS machine and respectively the instrument settings, slight modifications in the compound list – mostly of secondary metabolites – and addition of the starch digestion step described in Ref. [54]. The steps required for phytohormone analysis were omitted.

2.6.2. Sample preparation

Each 10 mg±1 freeze-dried duckweed sample was aliquoted into 1.1 cm^3 96-well Mini tubes (Axygen) and homogenized in extraction buffer containing acidified methanol (MeOH:water:formic acid 15:4:1 v/v/v). The values represent the proximate levels of those compounds not including moieties that are bound to other compounds (therefore excluding e.g. amino acids incorporated into proteins). For soluble sugar analysis, an aliquoted sample of the extract was further diluted with 70 % MeOH containing sorbitol as internal standard. For free amino acid and secondary metabolite analysis via LC-MS/MS, another aliquot of the extract was diluted in an aqueous mix of isotope-labelled amino acids (algal amino acid mixture-13C–15N; Sigma-Aldrich). The remaining undiluted extract was used for secondary metabolite analysis via HPLC-PDA. For starch analysis, the sample pellets were re-extracted twice with 50 % ethanol (EtOH) at 80 °C, then resuspended and diluted in water, and incubated at 98 °C to gelatinize the starch, which was then digested using an enzyme mix containing amyloglucosidase and alpha-amylase overnight at 37 °C. Glucose monomers of the starch were eluted during the digestion into the aqueous phase, and subsequently diluted with 70 % MeOH containing sorbitol as internal standard.

2.6.3. LC-MS/MS measurement

Metabolite analysis was done on a Shimadzu Nexera X3 LC-System connected to a Shimadzu LCMS-8060 mass spectrometer. For soluble sugar and starch analysis the LC system was equipped with an Agilent 1290 infinity II inline filter (0.3 μm) and an apHera™ NH2 column (150 × 4.6 mm, 5 μm; Supelco). The mobile phase comprised 0.1 % acetonitrile (Fisher Chemical) in water as Solvent A and acetonitrile (Fisher Chemical) as Solvent B in gradient mode. For free amino acid and secondary metabolites, the LC system was equipped with an Agilent 1290 infinity II inline filter (0.3 μm) and a ZORBAX Rapid resolution high definition (RRHD) Eclipse XDB-C18 column (3 × 50 mm, 1.8 μm; Agilent Technologies). The mobile phase comprised 0.05 % formic acid (Fisher Chemical), 0.1 % acetonitrile (Fisher Chemical) in water as Solvent A and MeOH (Fisher Chemical) as Solvent B in gradient mode. The mass spectrometer was equipped with an Electrospray ionization (ESI) source and was operated in multi-reaction-monitoring (MRM) mode. The gradient program and column oven settings for the chromatographic separation, as well as the ESI- and MRM-settings were used as described by Ref. [54], with addition of some further secondary metabolites to method 1A.

2.6.4. HPLC-PDA measurement

Analysis of flavonoid contents was done on a Shimadzu Nexera XR liquid chromatography (LC)-System equipped with an EC 4/3 Nucleodur ® Sphinx Reversed phase (RP) pre-column (5 μm, Macherey-Nagel) and a Nucleodur ® Sphinx RP column (250 × 4.6 mm, 5 μm, Macherey-Nagel). The mobile phase comprised 0.2 % formic acid (Fisher Chemical), 0.1 % acetonitrile (Fisher Chemical) in water as Solvent A and acetonitrile (Fisher Chemical) as Solvent B in gradient mode. Measurement was performed with a PDA detector.

The gradient programs and column oven settings for the chromatographic separation, as well as the detector settings and absorption wavelength were used as described by Ref. [54] (method 1D) with addition of luteolin (absorption wavelength 348 nm, retention time 17, 880 min) and apigenin (absorption wavelength 337 nm, retention time 19,675 min).

2.6.5. Analysis of metabolite data

Metabolite analysis was performed with the LabSolutions software (Version 5.97, Shimadzu). Data were quantified based on internal and external standards, for LC-MS/MS and HPLC-PDA analysis, respectively. LC-MS/MS data of flavonoids, chlorogenic acid and shikimic acid were quantified based relative to isotopically labelled amino acid standard and are therefore reported as arbitrary units (AU). For all other data an absolute quantification is presented. The data were normalised to the dry weight (DW) of the extracted freeze-dried powdered plant material. Starch quantification was obtained in mg/g by multiplying glucose monomers with the molecular weight of anhydroglucose. Tryptamine was not detected in any duckweed and therefore excluded from analysis. Ecotypes were the same as reported in 2.5.4, with the omission of one *L. minuta* ecotype KS06A.

3. Results

3.1. Duckweeds have high prevalence of C5 and C6 volatile compounds

Duckweeds contain high amounts of five and six carbon (C5 and C6) 'green leaf volatiles' 1-penten-3-one, 1-penten-3-ol, hexanal, *cis*-2-pentanol and pentadecanal (Table 1, Fig. S3). Other C5 compounds include *trans*-2-pentenal, and the ketones 3-pentanone and 2,3-pentanedione. Other C6 compounds include 2-hexenal and the alcohols hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol (Table S4). Carotenoid-derived beta-cyclocitral and *trans*-beta-ionone are other noteworthy compounds.

Additionally, contents of 1-penten-3-one are higher in northern UK ecotypes, benzaldehyde is higher in southern ecotypes and 1,3-di-*tert*-butylbenzene is higher in ecotypes from high light intensity environments (Table S5). Furthermore, twenty-two other compounds varied significantly between duckweed species (Fig. 2, Table S4).

3.2. Lemna minuta displays the most decreased aromatic profile

The duckweed species and ecotypes used in this study, the coordinates of origin and environmental data for origins are given in

Table 1
Top five duckweed volatile compounds by amounts as detected by semi-quantitative SPME-GCMS.

Compound	Retention time	LRI	CAS number	Descriptor	Function
1-penten-3-one	7.098	1041	1629-58-9	Spicy, pungent, peppery	Wound response, fungal resistance, ripening [55–57]
Hexanal	8.639	1101	66-25-1	Green, fresh, grassy.	Antimicrobial, enhance shelf-life [58–60]
1-penten-3-ol	10.632	1175	616-25-1	Ethereal, horseradish, green	Wound response, fungal resistance, ripening [55–57]
cis-2-pentenol	14.839	1337	1576-95-0	Green, phenolic, nasturtium	Reduce insect attraction, released from intact and mechanically-damaged leaves [61]
Pentadecanal	30.246	NA	2765-11-09	Fresh, waxy	Antimicrobial [62,63]

Functions are derived from the following sources [55]: Fisher et al., 2003 [56] Gorman et al., 2021 [57] Moummou et al., 2012 [58] (Song et al., 1996) [59] El Kayal et al., 2017 [60] Dhakshinamoorthy et al., 2020) [61] Tang et al., 2012 [62] Venuti et al., 2022 [63] Togashi et al., 2007. Descriptors are derived from The Good Scents Company (thegoodscentscompany.com). LRI = Linear retention index. CAS = Chemical abstracts service.

Fig. S1 (and Tables S1 and S2). Duckweed ecotypes were grouped by species for comparison. *Lemna minuta* has the lowest quantities for a range of aromatic compounds compared to other species. *Lemna minuta* has less 'green' descriptor compounds including tridecanal, 1-octen-3-ol, *trans*-geranylacetone but also fewer negative 'pungent/fatty' compounds like 2-tetradecanal and tetradecanal than *L. minor* (Fig. 2, Fig. S4). *Lemna minuta* has 'other' descriptor compounds higher than *L. minor* including butanol,3-methyl and 2-ethylfuran but lower levels of compounds without aroma descriptors. *Lemna minor* has the highest quantities of 'green' compounds including heptanal, *cis*-3-hexen-1-ol and 1-octen-3-ol. Pyrolle has a nutty aroma and was the only compound higher in *L. turionifera*. *Trans*-2-hexen-1-ol and 2-hexenal 'fresh and 'green' aromas are higher in *S. polyrhiza* than in *Lemna* (Fig. 2. Fig. S4A). Levels of VOCs also vary between individual ecotypes within a species but *L. minuta* ecotypes show the greatest consistency in profiles by clustering (Fig. S4B).

3.3. Duckweed volatile composition is similar to spinach and dandelion

Duckweed ecotypes are grouped by species and volatile profiles compared with other fast-growing herbs and leafy green vegetables. Comparisons of compounds common between duckweed and other herbs are given in Table S6. Duckweeds, dandelion, spinach and coriander have a comparable number of aromatic compounds; however basil contains more than double the number of total compounds at the same detection threshold, supported by its strong aroma. Duckweeds contain more hexanal and 'green', 'ethereal' C5 compounds: 3-pentanone, *trans*-2-pentenal, *cis*-2-pentenol, penten-3-one and 1-penten-3-ol than coriander or basil. Instead, basil and coriander have more 'woody' compounds including naphthalene- and sesquiterpenes with additional terpenes in basil (Table S6A). To detect differences between duckweed species, 32 compounds with concentrations <1 μg/kg in duckweeds were removed from analysis.

Duckweeds have the most similar aromatic profiles to spinach and dandelion, and are dissimilar to basil and coriander (Fig. 3A and B, Table S6). Duckweeds are higher than dandelion for 'ethereal' and 'pungent' aromas including 3-pentanone, naphthalene and 2-methyl naphthalene compared to higher 'minty', 'fresh', 'cheesy' and 'woody' aromas associated with dandelion. Nine compounds were found in duckweeds but not in spinach, with duckweed having higher 'green', 'fresh' and 'ethereal' positive descriptor compounds.

3.4. Penta-volatile compounds were frequently detected by participants

GC-O was conducted to identify key aroma compounds associated with duckweed (*L. japonica* KS18). Participants frequently identified fifteen compounds during headspace extraction, these are shown in Fig. 4. These include C5 alcohols and aldehydes, notably 1-penten-3-ol and beta-cyclocitral which are reported as odour active by all participants. Some compounds extracted from the headspace at similar run times and were hard to resolve, e.g. 1-pentanol with *cis*-4-heptanal (Fig. 4). Common aromatic compounds identified by the panel and their aroma descriptors are summarized in Table 2 to indicate 'pleasant' and 'malodourous' smells.

Of the 15 volatile compounds found in the duckweed species studied, only 7 have an odour activity value (OAV) high enough to significantly contribute to aroma profile of duckweed (Table 2). Butanal-3-methyl and tridecanal have the highest OAVs and 1-penten-3-ol and beta-cyclocitral have relatively low OAVs. The main positive aroma descriptors for *L. japonica* KS18 is a mixture of 'green', 'fruity', 'fresh' and on the negative end of the scale 'waxy', 'fatty' and 'oily' descriptors are common. Similar descriptors were identified between SPME-GCMS and GC-O for common duckweed VOCs (Table 1, Table 2, Fig. 4). Those odour active VOCs identified by participants in *L. japonica* including *trans*-geranylacetone, tridecanal, pentadecanal and butanal,3-methyl, are compounds which were significantly different in *L. minuta* compared to other species, therefore human perception of different species is expected to differ.

3.5. Spirodela and Lemna duckweed species have different free amino acid profiles but limited differences in sugar content

Duckweeds show a complete profile of free amino acids for human consumption, but show inter-species differences when grown in a common glasshouse environment. *Lemna minuta* and *S. polyrhiza* show decreased levels of the essential amino acids histidine and tryptophan compared to *Lemna minor*. In *Spirodela polyrhiza,* the aromatic amino acid precursor, shikimic acid is higher than *Lemna* species but otherwise *S. polyrhiza* displays lower levels of aromatic amino acid levels (Fig. 5). In *Lemna* and *Spirodela* duckweeds, the predominant sugar storage is starch, with lower levels of soluble sugars glucose and fructose. Sucrose levels were the lowest sugar detected but show a comparable average between species (Table 3). Sugar content is not significantly different between species but shows high variation between ecotypes and replicates.

3.6. Flavonoids are dominant in Spirodela polyrhiza compared to Lemna species

Spirodela polyrhiza in contrast to the four *Lemna* species is highly abundant in cyanidine- and chlorogenic-compounds, apigenin, luteolin and apigenin/luteolin 7-O-glucoside forms (Fig. 6, Fig. S5). *Lemna turionifera* has comparable luteolin 8-C-glucoside and more apigenin 8-C-glucoside than *S. polyrhiza*. Interestingly, these flavonoid compounds are low and not detected in other *Lemna* species. These findings were consistent between HPLC-PDA (Fig. S5) with LC-MS/MS (Fig. 6) using multiple ecotypes within species. *Lemna* species do however show higher levels of free amino acids compared to *Spirodela polyrhiza* (Figs. 5 and 7).

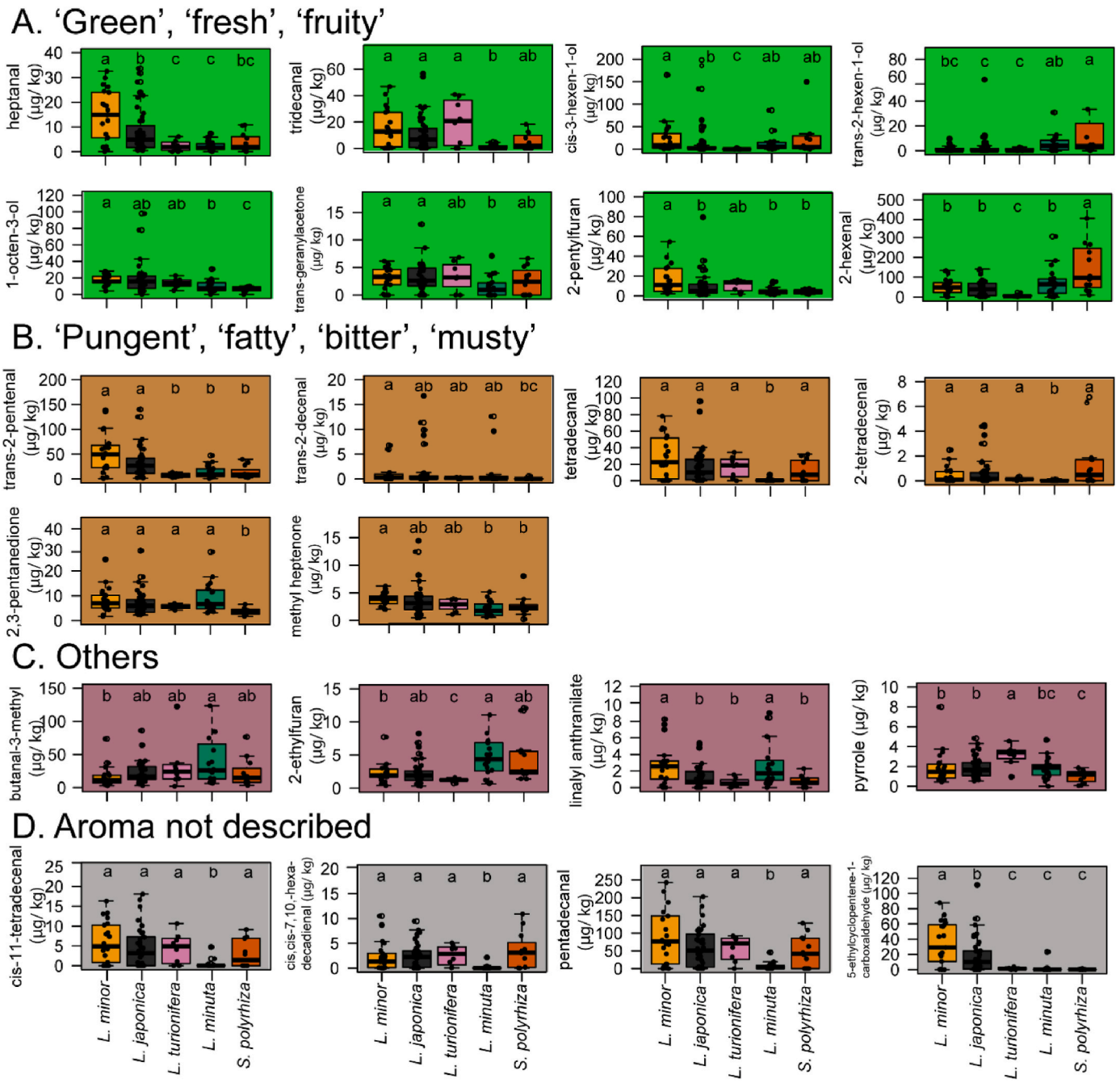

Fig. 2. Duckweed species had significantly different amounts of twenty-two aromatic compounds. Different aroma compounds found in duckweed freeze-dried powder grouped by species. *A*. Compounds with 'green', 'fresh' and 'fruity' positive descriptors. *B*. Compounds with 'pungent' 'fatty' 'bitter' and 'musty' negative descriptors. *C*. Others unique aroma compounds 'ethereal', 'chocolate', 'nutty'. *D*. Compounds lacking aroma data. Plots show median and 25 % and 75 % percentiles of concentrations of VOCs in µg/kg. Letters indicate significant differences for species by Paired Wilcoxon test using P = <0.05.

3.7. Analysis of duckweed growth using an ASD fieldspectrometer

Fresh and freeze-dried biomass for each ecotype is given in Table S7 and in Fig. S6. However the development of high-throughput detection methods will be useful to assess growth, physiological status and composition of duckweed to select new crop varieties. Green area was quantified by splitting photos into RGB channels as a measure of growth, and results are shown in Fig. S6. Hyperspectral vegetation indices were derived from an ASD fieldspectrometer in order to detect plant status (Fig. 1). Other vegetation index (OVI) has been used to estimate Nitrogen status in plants, and green model (GM), green index (GI) and normalised difference vegetation index (NDVI) used to predict greenness and plant health. All three greenness estimation parameters NDVI, GM and GI show an R^2 between 0.7 and 0.8 showing strong positive correlations with each other and with green area from RGB values of images.

Green model (GM) has moderate positive correlation with a range of amino acids (Fig. 8). OVI correlates positively and strongly with phenylalanine, histidine and ethanol content and total fresh weight biomass (FW) after six months growth (Fig. 8B and C). Vegetation indices cluster adjacent to health, growth and amino acid contents and opposite to sugar contents on a PCA biplot (Fig. 8D). Therefore GM and OVI could be useful detection methods for growth, biomass and nutritional quality.

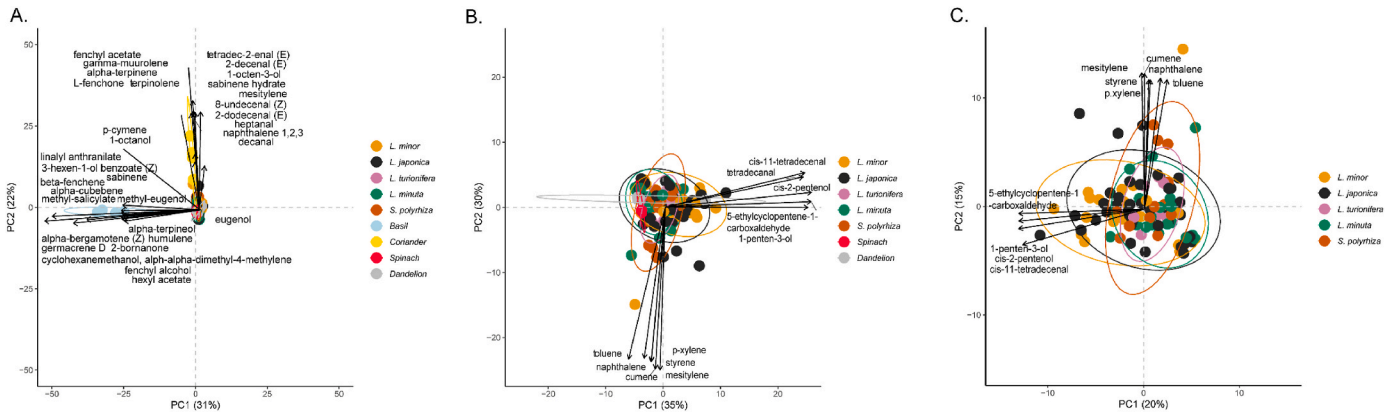

Fig. 3. Duckweeds have similar volatile profiles to spinach and dandelion. *A.* Basil and coriander form diverse clusters dissimilar to duckweeds using 92 compounds. Principal component analyses (PCA) for 92 compounds condensed onto two axis and coloured by five duckweed species and four herbs: basil, coriander, spinach and dandelion. *B.* Duckweed species do not differentiate from spinach and dandelion using 59 VOCs, after removal of low quantity compounds (>1 μg/kg) in duckweed. *C. Lemna japonica* and *Lemna minor* cluster away from *S. polyrhiza*. PC1 and PC2 account for approx. 50 % of the VOCs profile data variation. The VOCs contributing most to data variation are plotted with a cos2 value set at >0.7 using arrows to show direction of contribution.

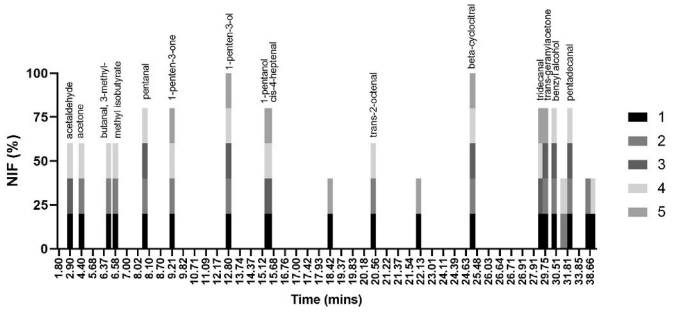

Fig. 4. Fifteen volatile compounds in duckweed were detected frequently by participants. An aromagram depicting aromatic compounds detected by GC-O as a time series and coloured in greyscale for each of the five participants. Nasal intensity frequency (NIF) is given between 0 % (not smelt) and 100 % is smelt by five participants. Aromatic compounds >50 % are indicated as frequently detected.

4. Discussion

4.1. Volatile profiles

Duckweed species not traditionally considered for human consumption were compared in a large-scale growth experiment for aroma perception to identify UK species with potential acceptability and usability in local food systems. Here we defined common aroma compounds in duckweeds, identified variation in amounts of VOCs within the *Lemna* and *Spirodela* species and found consistency between positive descriptor compounds identified with SPME-GCMS and those perceived by humans using GC-O. Aroma profiles of duckweeds were compared with those of commonly consumed leaf crops.

4.1.1. Volatile profiles of duckweed species had promising descriptors for food applications

Flavourings which are responsive to human taste includes sweet, sour, salty, bitter and savoury, which can be perceived as aromas by retronasal and orthonasal olfaction and are ultimately involved in decision-making regarding consumption and food likeliness [64]. Moreover, there is an innate preference for more 'sweet' smelling and tasting foods in human infants [65]. Human participants used

Table 2
L. japonica volatile compounds detected by GC-O with corresponding compound odour frequencies, odour activity values and descriptions.

Compound	Retention time	Concentration (μg/kg)	Odour threshold	Odour activity value	Descriptors
Acetaldehyde	2.90	5.86	120	0.05	**Pungent** ethereal aldehydic *fruity*
Acetone	4.40	5.62	500,000	0.00	Solvent ethereal *apple pear*
butanal,3-methyl-	6.48	13.98	2	69.91	Ethereal aldehydic chocolate *peach* **fatty**
methyl isobutyrate	6.58	12.15	7	1.74	*Fruity* floral *apple pineapple*
pentanal	8.06	16.58	42	0.39	**Fermented** bready *fruity* nutty *berry*
1-penten-3-one	9.21	9.02	1.3	6.49	Spicy **pungent** peppery mustard garlic onion
1-penten-3-ol	12.80	37.42	400	0.09	Ethereal horseradish *green* radish chrysanthemum vegetable *tropical fruity*
1-pentanol	15.28	8.88	4000	0.00	Fusel **fermented oily** sweet balsamic
cis-4-heptanal	15.38	0.89	0.8	1.12	**Oily fatty** *green* dairy milky creamy
trans-2-octenal	20.46	3.29	3	1.10	**Fatty** fresh cucumber *green* herbal *banana* **waxy** *green* leafy
beta-cyclocitral	25.29	0.42	5	0.08	*Tropical* saffron herbal clean rose sweet tobacco *green fruity*
tridecanal	28.95	0.55	0.01	55.33	Fresh clean aldehydic **soapy** *citrus* petal **waxy** *grapefruit peel*
trans-geranylacetone	29.75	0.69	60	0.01	Fresh *green* fruity **waxy** rose woody magnolia *tropical*
benzyl alcohol	30.33	0.11	10,000	0.00	Floral rose phenolic balsamic
pentadecanal	33.05	8.05	1	8.05	Fresh **waxy**

[a] Odour frequencies for compounds were retrieved from Ref. [51] and descriptors from The Good Scents Company [52]. Compounds with positive 'green' and 'fruity' descriptors are highlighted in italics. Descriptors in bold are considered to have negative associations. Compounds with odour activity values (OAV) > 1 are marked in grey and are expected to contribute most to human aromatic perception.

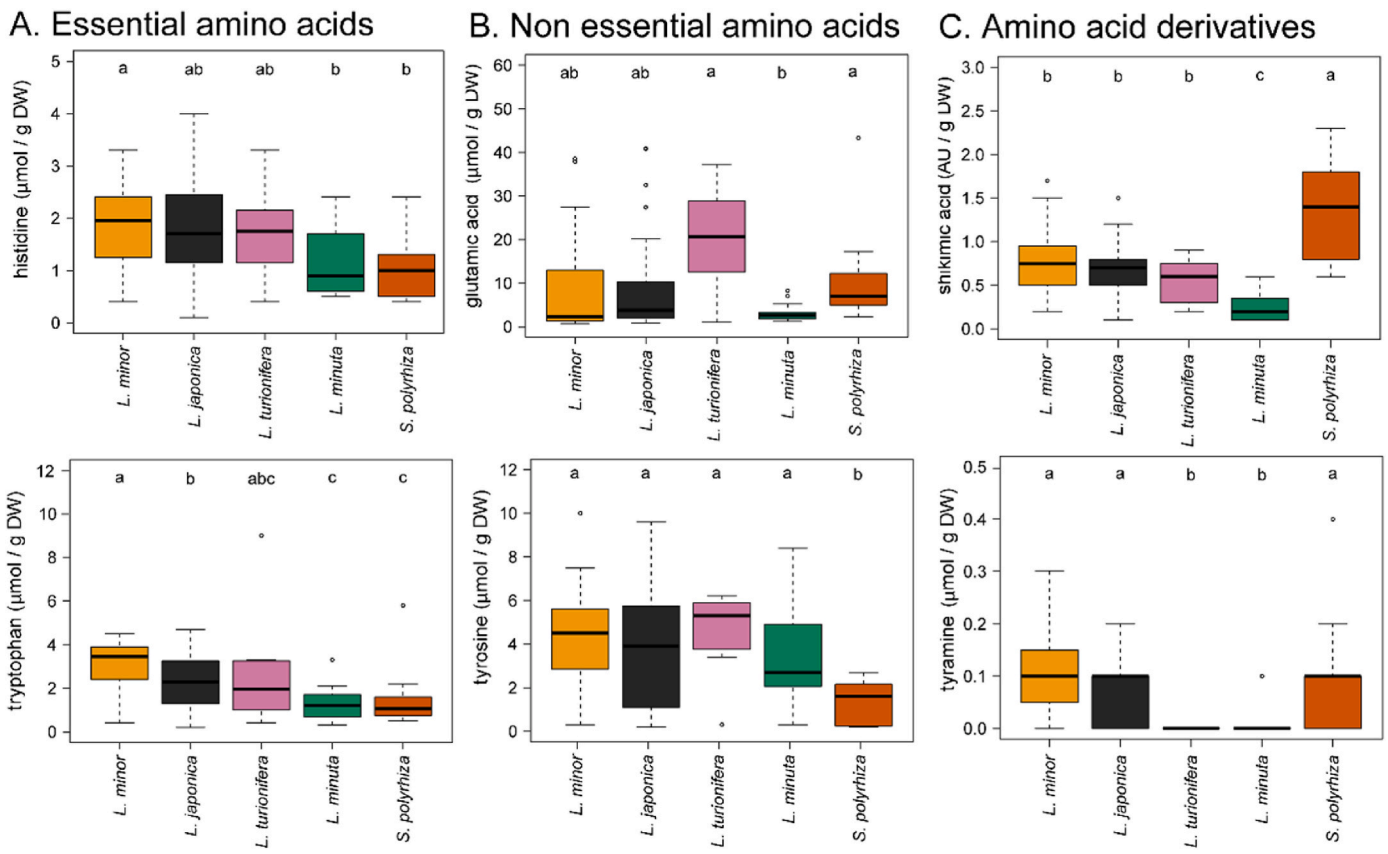

Fig. 5. ***Spirodela polyrhiza*** **has decreased essential amino acid profiles compared with** ***Lemna****. A:D.* Metabolite contents measured in duckweed species normalised to duckweed dried weight and presented as µmol/g DW or AU/g DW. *A*. essential amino acids, *B*. non-essential amino acids and *C*. amino acid precursors/derivatives. Plots show median values with 75 % and 25 % percentiles. Letters indicate significant differences between species from Kruskal-Wallis and post-hoc paired Wilcoxon test P = <0.05. *S. polyrhiza* and *Lemna minuta* have 1–2 fold lower histidine and tryptophan compared to *L. minor*. *Spirodela polyrhiza* species show 3-fold reduction in tyrosine but shikimic acid is 5-fold higher than *Lemna* species. *Lemna minuta* is 2–6 fold lower in glutamic acid compared to other species, with the highest levels in *L. turionifera*. Both *L. turionifera* and *L. minuta* have 18-fold reduction in tyramine compared to other duckweed species.

Table 3
Sugar content shows high variation within duckweed species.

Sugar	Average concentrations (µmol/g DW or mg/g DW for starch)				
	L. japonica	*L. minor*	*L. minuta*	*L. turionifera*	*S. polyrhiza*
glucose	57.02	42.76	45.26	72.84	50.53
fructose	70.93	47.16	62.68	94.38	94.94
sucrose	4.63	0.19	2.25	1.59	7.73
starch	99.65	126.04	154.84	150.43	101.12
Sugar	Range of concentrations (µmol/g DW or mg/g DW for starch)				
	L. japonica	*L. minor*	*L. minuta*	*L. turionifera*	*S. polyrhiza*
glucose	3.2–539	17.3–209.3	16.8–167.4	18.6–298.7	22.7–126.9
fructose	6.2–580.7	17.1–262	16.5–366.3	21–429	11.6–433.5
sucrose	0–36.4	0–0.7	0.1–27.5	0.1–11.6	0.1–28.7
starch	17.2–346.1	16.7–477.6	10.8–369.5	25.2–323.9	4.8–310.5

[a] Concentrations of sugars measured in multiple ecotypes within duckweed species by LCMS/MS. For each species, all raw values are presented as averages and ranges. Soluble sugars were measured in µmol/g DW and starch is presented in mg/g DW. Individual ecotypes within each species group were $n = 10$ *L. japonica*, $n = 5$ *L. minor*, $n = 4$ *L. minuta*, $n = 2$ *L. turionifera*, $n = 3$ *S. polyrhiza* and each ecotype was replicated four times positioned around a glasshouse. A Kruskal-Wallis test was used to derive significant differences between species averages with P = <0.05.

descriptors including 'green', 'fruity' and 'floral' for the scents of *L. japonica* during orthonasal sensory analysis (GC-O). Coincidently these descriptors are most correlated with the appealing perception of 'sweet' flavour [66] and 'green', 'grassy' and 'floral' descriptors are used generally to describe the aromas of other dietary vegetables available for consumption [67].

The negative descriptors associated with duckweeds included 'bitter' and 'pungent' which have also been descriptors associated with sulfur-containing nitriles, aldehydes and alcoholic compounds in green vegetables, for example broccoli [68]. No severely negative descriptors such as 'cheesy', 'eggy', 'fishy' or 'rotten' were found in freeze-dried duckweed. Therefore, UK-sourced species could be an acceptable novel vegetable depending on amounts, ratios and interactions between aroma compounds.

It is noteworthy that processing of foodstuffs can affect volatile profiles and aroma perception. For example, freezing duckweed

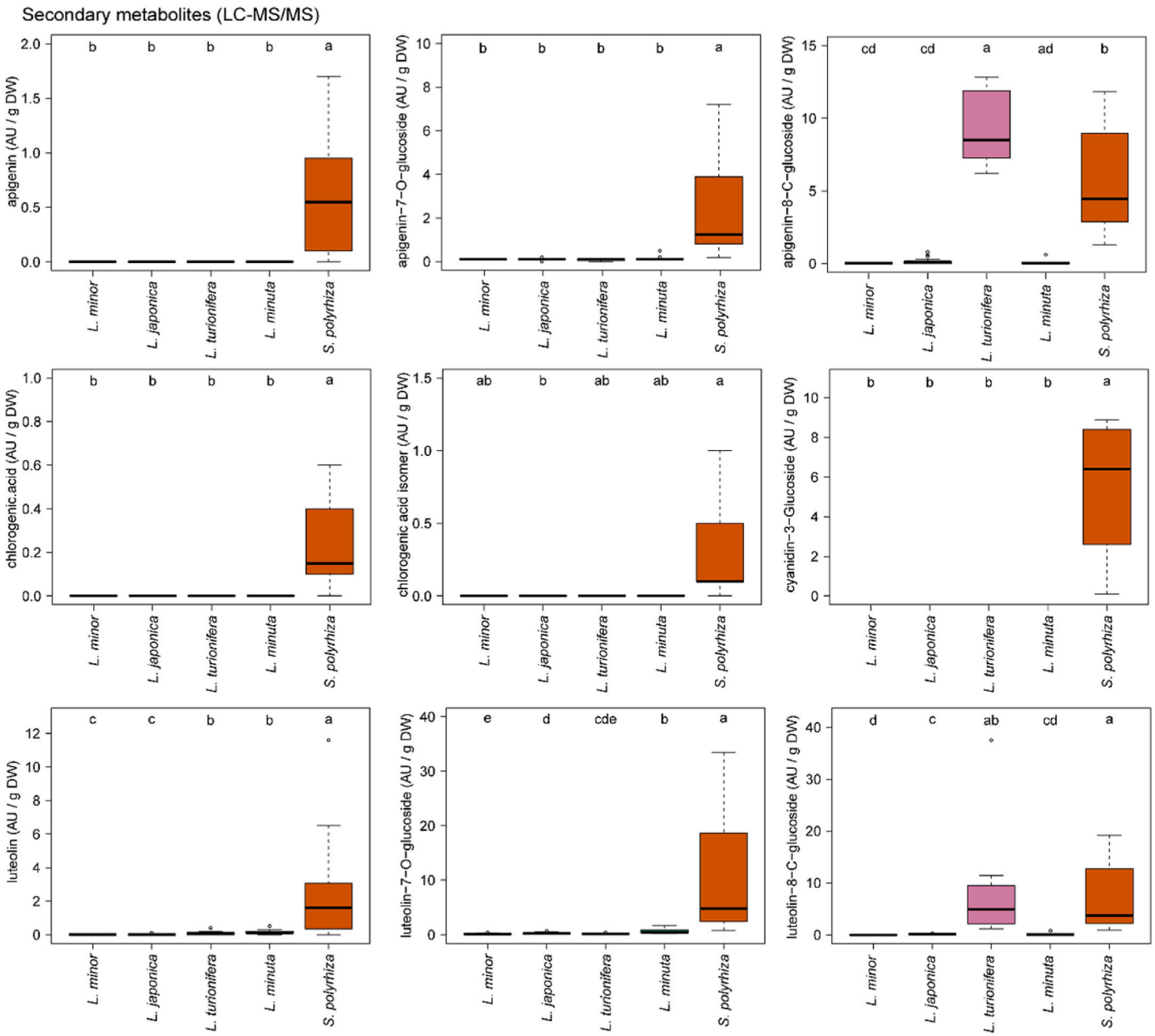

Fig. 6. Secondary metabolites including flavonoid compounds are dominant in *S. polyrhiza* compared to *Lemna* species. Boxplots indicating relative amounts of polyphenols including flavonoids in duckweed species (in AU/g DW). Boxplots are arranged by compounds alphabetically with conjugated forms next to their corresponding base compound in each row. Plots show median values and 25 % and 75 % percentiles and are coloured by species groupings. Letters indicate significant differences by Wilcoxon paired statistic <0.05.

concentrate increased bitterness and decreased protein content [69], and drying under direct sunlight decreased beta-carotene [70], which are volatile precursors for apocarotenoids such as beta-cyclocitral. Fresh duckweed may show differences in odour active compounds from that found in freeze-dried duckweed here. However, freeze-drying maintains high quality of many herbs and spices and preserves phenolic contents compared to other preparation methods [71]. It is also in line with the development of duckweed freeze-dried powders for the health and protein supplement markets.

Short chain volatile compounds (penta- hexa- and hepta-) in duckweeds are similar to other leafy vegetables, like broccoli and in tomato and olive fruits [72–74]. Duckweeds have higher amounts of 'green' and 'fresh' descriptive compounds than basil, coriander and spinach overall. *Lemna minor* and spinach had equal acceptability as inputs in foodstuffs in human feeding trials [75], perhaps because of their similar aroma profiles.

From preliminary olfactory analysis of a *L. japonica* duckweed sample, the most frequently detected compounds were beta-cyclocitral and 1-penten-3-ol (Fig. 4), the latter was also one of the compounds with the highest abundance from GCMS (Table 1, Fig. S3). Despite this, these were indicated to not have significantly high OAVs to be detected by humans. Additionally, beta-cyclocitral is reported as a substance with high anosnia, where ~34 % participants are not expected to smell it [76]. In contrast, hexanal was highly abundant but surprisingly not detected amongst the duckweed sensory panel, despite previous detection in other vegetables and salad crops [77]. Additional compounds without peaks in GCMS were found to contribute to human perception of aroma from GC-O in duckweed, due to low odour detection thresholds [78,79]. As aroma compounds vary between species they are likely to vary too in both human acceptability and storage potential.

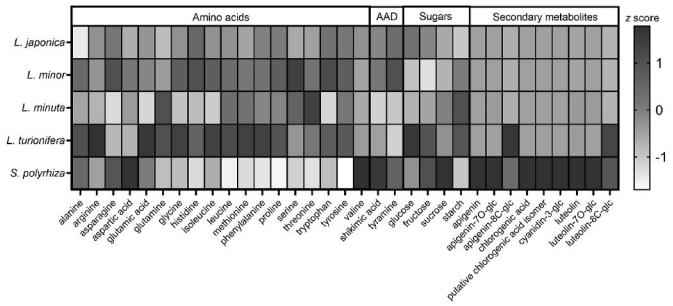

Fig. 7. *Spirodela* is higher in flavonoid secondary metabolites than *Lemna* species but has decreased free amino acids. Heat map with false colour greyscale for average metabolite abundance per dry weight of freeze-dried duckweed powder. Metabolites include free amino acids, amino acid precursors/derivatives (ADD), sugars, starch and secondary metabolites measured in ecotypes of five duckweed species. *z* scores for each compound were calculated using standardisation to the mean and SDs for the whole sample size. Compounds were measured by LC-MS/MS.

Lemna minor have the highest range and more 'green' and 'fruity' descriptors of the included *Lemna* species. Decreased levels of 'green' aroma compounds in *L. minuta and L. turionifera* and more compounds with 'other' descriptors has likely outcomes for uniqueness of their aroma profiles. *Lemna minuta* had a distinct aroma profile with decreased numbers of several aromatic compounds, including those smelt by participants of the *L. japonica* sample (*trans*-geranylacetone, tridecanal, pentadecanal) but higher presence of butanal, 3-methyl, a compound with the highest OAV attributing an 'ethereal' aroma.

4.1.2. *The role of VOCs in the storage potential of duckweeds*

Fresh-stored duckweed is reportedly unspoiled for 28 days [80,81] supporting general opinion of good longevity in post-harvest storage, possibly due to high phenolic contents. Furthermore, duckweed extracts show antibacterial activity against food spoilage microorganisms including *Bacillus*, *Staphylococcus* and *Pseudomonas* species [33,34]. Supporting this, incorporation of *Lemna minor* extract into beef burgers decreased protein oxidative products from meat [35] and application into polyvinylalcohol packaging limited fungal spoilage during storage of avocados [82]. The five highest VOC contents in duckweed here are recognized compounds with antimicrobial functions, possibly contributing to extending shelf-life (Table 1). Moreover, hexanal and heptanal provide fungal resistance in other plants [60,83], and are produced in varying amounts by duckweeds. Further exploration of the roles of specific VOCs and phenolics in post-harvest storage are required in the future.

4.2. *Metabolites in duckweed for food applications*

Here we show UK duckweed species grown under common

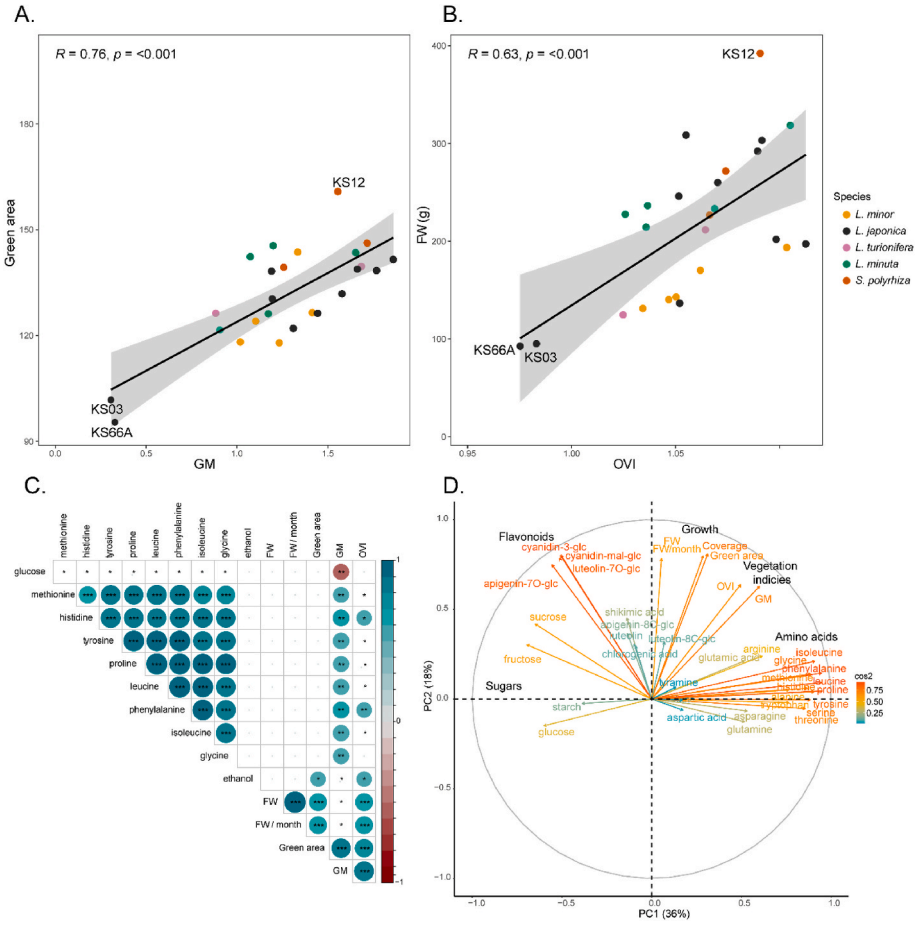

Fig. 8. Vegetation indices can be used as a proxy for health, growth and metabolite profiles of duckweeds. *A.* Linear model showing positive correlation between ASD fieldspectrometer derived GM values with green area as obtained by RGB from photographs at four weeks growth averaged by ecotypes. *B.* Linear model showing correlation between OVI measured by ASD fieldspectrometer at four weeks growth with total fresh weight at six months averaged by ecotypes. Points are coloured by species and outlying individuals are labelled. *C.* Correlation plot matrix of significant relationships by Pearson's correlation co-efficient of amino acids, sugars, growth, health and vegetation indices. *D.* PCA biplot for variable relationships showing trade-offs for growth/amino acid content and sugars/flavonoids.

glasshouse conditions have different nutritional potentials relevant in food applications.

4.2.1. *Spirodela is dominant in flavonoids compared to Lemna species*

Our work shows species-dependent variation in beneficial secondary metabolite contents of duckweed. *Lemna minor* was identified with potential as a future food due to a range nutritional qualities including higher composition of the polyphenol naringenin compared to a *Wolffia* species [84]. Here, *Spirodela polyrhiza* has a higher polyphenol content and greater number of different flavonoid polyphenolic compounds compared to four *Lemna* species (Figs. 6 and 7). *Spirodela* is high in cyanidine-3-glucoside, chlorogenic acid and shikimic acid which are supposedly anti-inflammatory and anti-cancer compounds. Luteolin and apigenin and their 7-O- and 8-C-gluc. conjugates are abundant in *Spirodela* [85] acting as antioxidants and may even contribute to anti-tumour and anti-inflammatory medical properties of *Spirodela* [25,26,30]. Chlorogenic acid was previously found in *Spirodela* but not in *Landoltia* or *Wolffia* species [31], the lack of detection in *Lemna* provides supporting evidence that it may be exclusive to the *Spirodela* genus of duckweeds.

4.2.2. *Spirodela contains less free amino acid compositions compared to Lemna*

In contrast to its higher flavonoid content, *S. polyrhiza* has decreased contents of three free amino acids compared to *Lemna* species. Amino acids are precursors for certain secondary metabolites, such as flavonoids. Therefore, the flavonoid biosynthesis in *S. polyrhiza* might lead to a reduction of free phenylalanine and related amino acids. *Lemna minor* has the highest levels of the essential amino acids tryptophan and histidine, and displayed previously higher amounts by dry weight than soya, rice and wheat [86]. However, both tryptophan and histidine impart bitter flavours in mushoom species and may contribute to increased bitterness of *L. minor* in comparison with *L. minuta* and *S. polyrhiza* [87]. *Lemna turionifera* had more free glutamic acid and arginine than other species (Figs. 5 and 7), with glutamic acid one of the highest contributing free amino acids to flavour, imparting savoury or satisfactory tastes, whilst arginine contributes heavily to bitterness [88]. Further analysis is required to assess which species show the most promise for providing 'complete' amino acid composition for human nutrition [89,90] with the lowest trade-off in bitterness possible.

4.3. *Relationship between metabolites and growth for optimisation for food production*

Growth rate potential is important when selecting duckweed ecotypes for commercial purposes. In this context the cost to the plant of synthesizing secondary compounds and amino acids may need to be considered too, creating a complex trade-off. Starch content is not a good indicator of growth rate in duckweed, as it is in staple cereal grains [91]: the negative relationship between growth and starch content here and in Sree & Appenroth (2014) [92] indicates a stress response or a lack of ability to utilise storage sugars in growth. A closer relationship between flavonoids and sugar content was seen here (Fig. 8D), possibly because of conjugation of flavonoids through glycosylation.

4.4. *Optimisation of duckweed ecotypes for food production*

For commercial applications as either a fresh vegetable herb or dried protein supplement, high and consistent yields (dry and fresh weight) are required. Biomass showed variability between a trio of harvests conducted over a six month period here (Fig. S6C:F), and previously [93], so the conditions required for predictable harvests represents an ongoing challenge for duckweed development as sustainable food. However, we conclude that *Spirodela polyrhiza* ecotype KS12 has the highest greenness values, surface coverage and biomass with the benefits of high flavonoid content (Fig. 8A,B and Figs. S5 and S6). Additionally, this work recognizes indoor duckweed production as being intensive i.e. requiring high resources [70] and supports a drive towards automation to monitor growth, water and nutrient supply and harvest.

5. Conclusion

Wolffia species are commonly utilised for human consumption worldwide, with additional recent inclusion in spaceflight missions [94,95]. Here we recognize *Lemna* and *Spirodela* species, which also show positive sensory and nutritional properties. Specific *Spirodela* and *Lemna* species may be suitable for food applications based on aroma, flavonoid content and free amino acid composition. In future, wider human feeding studies and digestion assays with *Lemna* and *Spirodela* species should be conducted to assess taste and mouthfeel, any acceptability issues, and negative effects from duckweed consumption.

Future studies measuring stability of VOC profiles during processing methods, together with antimicrobial activity and storage potential of specific species are required. Future aims include isolation of genetic components to increase compounds associated with positive aroma. Concordantly, to increase acceptability, mitigating the few identified negative aroma traits should be a goal akin to that performed in tomato and wheat [96,97]. Future selection of other underutilized duckweed species and ecotypes should use high-throughput techniques to detect high growth in parallel with '-omics' technologies for nutritional assessment. This work supports the use of digital media to educate the population about food research [98], in particular here the aroma profiles and potential benefits of different duckweed species as a novel food.

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CRediT authorship contribution statement

Kellie E. Smith: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Martin Schäfer:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Mui Lim:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Data curation. **Carlos A. Robles-Zazueta:** Writing – review & editing, Resources, Data curation. **Laura Cowan:** Writing – review & editing, Data curation. **Ian D. Fisk:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. **Shuqing Xu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization. **Erik H. Murchie:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Data availability

The sequences for duckweed genomes in this panel are deposited under project codes PRJNA1026139 and PRJNA1074359 on the NCBI Sequence read archive (SRA). Scripts used for analysis are available at: https://github.com/Duckweed-KS/Flavour_metabolites

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101263.

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