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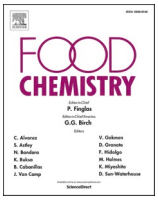

Metabolomics and sensory evaluation of white asparagus ingredients in instant soups unveil important (off-)flavours

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ABSTRACT

Split-stream processing of asparagus waste stream is a novel approach to produce spray-dried powder and fibre. Asparagus ingredients processed by this method and a commercial asparagus powder were compared by evaluating their flavour profile in a soup formulation. Professional sensory panel and untargeted metabolomics approaches using GC–MS and LC–MS were carried out. Unsupervised and supervised statistical analyses were performed to highlight discriminatory metabolites and correlate these to sensory attributes. The spray-dried powder scored higher on asparagus flavour compared to the commercial powder. The fibre negatively impacted the taste and mouthfeel of the soups. GC-O-MS confirmed the role of dimethyl sulphide, 2-methoxy-3-isopropyl pyrazine and 2-methoxy-3-isobutyl pyrazine in asparagus odour. Seven new volatile compounds are also proposed to contribute to asparagus flavour notes, most of which were more abundant in the spray-dried powder. This research demonstrates the feasibility of upcycling asparagus waste streams into flavour-rich ingredients with good sensorial properties.

1. Introduction

Instant vegetable soup is primarily composed of dried vegetable powders. Commercial vegetable powders are commonly made by oven-drying (i.e. hot-air drying) small vegetable pieces which are then milled into a fine powder (Kamiloglu, Toydemir, Boyacioglu, Beekwilder, Hall, & Capanoglu, 2016; Karam, Petit, Zimmer, Baudelaire Djantou, & Scher, 2016). This drying process is known to irreversibly alter the flavour profile of the vegetable (Nijhuis et al., 1998; Sagar, Suresh Kumar, & Suresh Kumar, 2010). Consequently, supplementary flavour components often need to be added to the dried vegetable powders to obtain the desired sensory complexity of the final product (e.g. instant soups) and to ensure flavour stability. To produce products that are perceived as natural and healthy, the addition of these flavourings should ideally be avoided (Román et al., 2017).

A novel split-stream process has recently been developed to produce ingredients from vegetable waste streams generated during or after harvesting (Siccama, Pegiou, Zhang, et al., 2021). In this process, vegetable juice is separated from the fibre fraction and both streams are processed and dried separately, after which they can be recombined to yield vegetable powder. White asparagus (*Asparagus officinalis*) was selected as the model crop for two reasons. First, because the flavour of the commercial asparagus powders does not meet the standards of the industry and thus flavour supplements are required and second, due to the large waste volumes generated within a harvest season (ca. 30 % of all harvested material). A significant part of this waste stream comprises the basal parts of each asparagus spear (ca. 5 cm) which are cut off and discarded (Pegiou, Mumm, Acharya, de Vos, & Hall, 2020). Within the scope of a more sustainable and circular food system, it is of interest to investigate the potential of exploiting these vegetable waste streams to generate dried powders for use as food ingredients in products such as instant soups (Siccama, Pegiou, Zhang, et al., 2021). The composition of volatile compounds of the obtained spray-dried asparagus powder has previously been investigated and it was suggested as a potential new natural food ingredient with high retention of volatile flavour compounds (Siccama, Pegiou, Eijkelboom, et al., 2021), but yet its flavour

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profile still needs to be assessed concerning sensory attributes like aroma, taste and mouthfeel.

It has been demonstrated that combining sensory evaluation and metabolomics may give a deeper insights into the flavour composition of food products (Jacobs, van den Berg, & Hall, 2021; Utpott, Rodrigues, Rios, Mercali, & Flôres, 2022). Recently, few studies have used the potential of both sensory evaluation and metabolomics for examining the flavour of, e.g., strawberry (Buvé et al., 2018), beer (Bettenhausen et al., 2020), tomato soups (Davarzani et al., 2021), cocoa powder (Greño, Plaza, Luisa Marina, & Castro Puyana, 2023) and soy sauce (Diez-Simon, Eichelsheim, Jacobs, Mumm, & Hall, 2021). There have been three studies combining sensory and metabolomics techniques to study cooked white asparagus (Dawid & Hofmann, 2012; Hoberg, Ulrich, Gottwald, & Rosen, 2003; Ulrich, Hoberg, Bittner, Engewald, & Meilchen, 2001). It is thanks to these three studies that we can link specific steroidal saponins to the bitter asparagus notes (Dawid & Hofmann, 2012) and refer to several volatiles (e.g. dimethyl sulphide, 2-methoxy-3-isopropylpyrazine, hexanal) as being "key odorants" of cooked asparagus (Hoberg et al., 2003; Ulrich et al., 2001). Specifically, dimethyl sulphide (DMS), is considered to be the aroma compound giving the typical "cooked asparagus" flavour (Tressl et al., 1977a, 1977b; Ulrich et al., 2001). However, it is of relevance to confirm the contribution of known asparagus flavour compounds to the sensory profile of the processed asparagus materials as it is likely that multiple compounds are required to give the true asparagus experience.

The main objective of the study described here was to compare the in-house processed asparagus ingredients (i.e. concentrate and spray-dried powder) to a commercial asparagus powder with and without a flavour supplement, by evaluating the flavour profile in an instant soup formulation. The soup formulations were prepared based on the composition of a commercial instant asparagus soup only differing in the asparagus ingredients. We hypothesized that the variation in the ingredient composition of the soups would be reflected by differences in both the metabolite composition and the sensory profiles. The spray-dried powder was hypothesized to have a richer flavour profile compared to the commercial powder which is oven-dried. Moreover, the asparagus fibre, which remains after the extraction of the juice from the asparagus pieces, was processed and added to some of the soup prototypes. We hypothesized that the fibre would provide more structure and thickness to the soups, influencing the presumed mouthfeel sensation.

To investigate these hypotheses, the soup prototypes were assessed by a trained expert panel and were analysed using advanced metabolomics platforms. Solid phase microextraction gas chromatography mass spectrometry (SPME GC–MS) and liquid chromatography mass spectrometry (LC–MS) systems were employed to profile the volatile and non-volatile metabolites, respectively. Metabolite data were processed using an untargeted metabolomics approach. Random Forest techniques were used to link the sensory and metabolomics data. The odour-activity and aroma attributes of the highlighted volatile compounds were confirmed by GC Olfactometry MS (GC-O-MS). Physical properties (particle size distribution, viscosity and morphology) of the samples were also monitored in relation to the mouthfeel of the soups.

2. Materials & methods

2.1. Chemicals and reagents

Maltodextrin DE12 (GLUCIDEX® 12, Roquette, Lestrem, France) was used as carrier for the spray drying. A mix of *n*-alkanes (C_6 – C_{21}) was prepared. All alkanes were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The analytical standards used for metabolite identification had a purity between 96 and 99 %. All standards were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) except for methanethiol, 1,3-diethylbenzene, styrene and 1,4-diethylebnzene which were purchased from Greyhound Chromatography (Wallasey, UK). All standards were dissolved in methanol (Biosolve BV, Valkenswaard, The Netherlands). Methanol, formic acid and acetonitrile (Sigma-Aldrich, Zwijndrecht, The Netherlands) were used for the extraction and analysis of semi-polar non-volatile metabolites present in the soup samples.

2.2. Production of asparagus ingredients

Raw fresh asparagus cut-offs were kindly provided by Teboza BV (Helden, The Netherlands). The concentrated asparagus juice (concentrate) and spray-dried powder were prepared under hygienic conditions and following the split-stream processing method as described previously (Siccama, Pegiou, Eijkelboom, et al., 2021). In brief, concentrated juice was prepared from asparagus cut-offs by pressing them, followed by centrifugation of the juice to remove any solids. The juice was concentrated using reverse osmosis. The remaining asparagus fibre after pressing was dried with hot air and milled to fine powder.

Aliquots of the concentrate (21.7 % w/w solids) were transferred to 1 L autoclaved glass bottles and stored at −20 °C until further analyses. The remainder of the concentrate was used for spray drying after adding maltodextrin DE12 in a 1:2 mass ratio (asparagus solids: maltodextrin). A Mobile Minor spray dryer (GEA, Dusseldorf, Germany) was used for spray drying with inlet and outlet temperatures of 196 ± 3 °C and 85 ± 1 °C, respectively. The spray-dried powders were stored in sealed aluminium bags at ambient temperature.

The asparagus fibres were stored in sealed plastic bags at −20 °C until further processing. After storage, the fibres were thawed in boiling water and washed at least three times at 70 °C to remove traces of sand. Subsequently, they were blanched at 90 °C for 3 min and dried in a food dehydrator (Sedona, Tribest, USA) at 68 °C for 20 h. After drying, the fibres were milled using a Pulverissette-14 RotorMill (Fritsch, Idar-Oberstein, Germany) using a 0.2 mm sieve and at 10,000 rpm. The milled fibres were manually sieved using a 0.25 mm sieve and the fine fraction was stored in sealed aluminium bags at ambient temperature for later use as one of the ingredients for two soup prototypes.

To ensure food safe processing of the asparagus ingredients, a risk assessment was performed. Hygiene indicators were tested by taking samples during different steps in the process. The analysed indicators were total viable count, *Enterobacteriaceae*, *Lactobacilli*, yeast, moulds, *Bacillus cereus* and *Staphylococcus aureus*. The results are reported in the Table S1. Lastly, we ensured the elimination of microorganisms by heating the asparagus soups for at least 2 min at 70 °C before consumption (Cebrián, Condón, & Mañas, 2017).

2.3. Preparation of asparagus soup prototypes

Six asparagus soup prototypes were prepared for evaluation of the sensory and metabolite profiles. Their composition was based on the recipe of a commercial asparagus instant soup. All prototypes included the same concentration of potato starch, salt, asparagus solids and final maltodextrin content. The six prototypes differed regarding the asparagus ingredients: the commercial dried asparagus powder with (CF) and without (C) asparagus flavour mix, the juice concentrate with (JA) and without (J) asparagus fibre and the spray-dried powder with (SA) and without (S) asparagus fibre (Table 1). The commercial powder and asparagus flavour mix were kindly provided by Unilever (R&D Unilever BV, Wageningen, The Netherlands). Potato starch and sea salt were purchased from a local supermarket (Wageningen, The Netherlands).

The dry ingredients, i.e. starch, maltodextrin, salt and asparagus powders, were weighed according to Table 1 and mixed four days before the first sensory evaluation training session and stored in sealed plastic opaque containers at room temperature. The concentrate (1L flask) was aliquoted into 50 mL polypropylene screw cap test tubes (Sarstedt AG & Co, Germany) after thawing overnight at 4 °C and aliquots were stored at −20 °C until use. For each sensory evaluation, the required number of test tubes with concentrate were transferred to the fridge the day before

Table 1
Recipe for the preparation of the six asparagus soup prototypes in 1 L water. The labels indicate the asparagus ingredients of the soups. The amount of the asparagus ingredient is on total weight basis, maintaining the same asparagus solids content per soup. The salt, starch and final maltodextrin content were the same for all prototypes and based on the composition of a commercial product. The volume mean particle diameter (D[4,3]) and the viscosity of the soups measured at 50 s^{-1} and 40 °C (n = 2) are presented. The letters show the significant differences based on pairwise comparisons after one-way ANOVA with the Games-Howell post hoc test for unequal variances (significant p-value < 0.05).

Label	Asparagus ingredients	Primary asparagus component (powder or concentrate) (g)	Asparagus fibre (g)	Maltodextrin (g)	D[4,3] (μm)	Viscosity (mPa•s)
CF	Commercial powder and added flavour mix	7.15	0	41.54	214 ± 7 [a]	7.3 ± 4 [a]
C	Commercial powder	7.15	0	41.54	186 ± 48 [a]	8.4 ± 4 [a]
JA	Concentrate and asparagus fibre	14.31	3.58	41.54	227 ± 19 [a]	8.4 ± 0.5 [a]
J	Concentrate	28.62	0	41.54	184 ± 0 [a]	11.8 ± 6 [a]
SA	Spray-dried powder and asparagus fibre	10.84	3.58	35.55	223 ± 18 [a]	8.4 ± 1 [a]
S	Spray-dried powder	21.68	0	29.43	199 ± 32 [a]	5.8 ± 0.7 [a]

Note: 23.08 g of potato starch and 5.85 g of salt were added to 1 L of all soups.

and thawed to 4 °C. Shortly before evaluation, 1L of boiling water per prototype was added to the dry ingredients. In the case of 'J' and 'JA', the aliquoted concentrate was added to the dry ingredients immediately before adding the water. All samples were individually mixed using an electric hand-blender (Kenwood kMix Triblade Hand Blender, Kenwood Corporation, Tokyo, Japan) for 30 s. The samples were kept warm in Thermos flasks until the evaluation. When serving to the panellists, one additional sample (15 mL) of each soup was taken and stored in a 50 mL polypropylene screw cap test tube (Sarstedt AG & Co, Germany) at −20 °C for the metabolomics analysis.

2.4. Sensory evaluation

The sensory evaluation was carried out at the facilities of Essensor BV (Wageningen, The Netherlands). The sensory panel was selected from the top 10 % of the population after screening on sensory abilities and sensitivities following the ISO 8586 criteria. Ten selected professional panellists first became fully acquainted with the six soup prototypes during four training sessions, to be acquainted with the samples without knowing the composition of ingredients, before the final descriptive evaluation. The sessions were carried out on separate days and each started at 10:00 AM. A set of 24 attributes was determined for the soups during the four training sessions, which covered odour, taste, mouthfeel and aftertaste attributes (Table S2).

During the final descriptive sensory evaluation, the six soup prototypes were served to the panellists twice in randomized order. Each sample was labelled with a unique three-digit code. The serving temperature was 65–70 °C. The panellists first evaluated the soups on the odour attributes. Subsequently, each sample was tasted at 60 °C to evaluate the taste, mouthfeel and after-feel attributes. Each panel member used thermometers to confirm and monitor the temperature. Evaluation scores were within the range of 0–100.

2.4.1. Data analysis

For the analysis of the sensory profiling data, the SenPAQ© software (QIStatistics, UK) was used. Principal component analysis (PCA) was performed to investigate the variation in the sensory profiles of the soup prototypes, after normalisation of the raw data (mean-centred and divided by the standard deviation). Two-way Analysis of variance (ANOVA) was performed per attribute and p-values were adjusted for multiple comparisons using the Benjamin-Hochberg approach. The Quality Index (QI) technique (Hyldig & Green-Petersen, 2004) was applied as a measurement of whether the panellists could reliably distinguish the soup prototypes regarding each sensory attribute.

2.5. Metabolomics

To profile the volatile and non-volatile chemical composition of the six soup prototypes, 15 mL was taken from each soup on each training sensory session day as well as the final descriptive evaluation and stored at −20 °C. After the final descriptive evaluation was complete, all samples were transported to the lab on ice. Once fully defrosted, these were placed in a water-bath at 70 °C to mimic the temperature as served to the panellists. Afterwards, they were respectively aliquoted in glass vials and Eppendorf tubes for further analysis using SPME GC–MS and LC–MS as described below. All samples were analysed in a single sequence per metabolomics platform, in randomized order.

2.5.1. Analysis of volatile compounds

To profile the volatile compounds, 1 mL per soup replicate was pipetted in a 10 mL ND18 headspace screw glass vial (BGB®, Germany) and vials were closed with ND18 magnetic screw caps (8 mm hole) with Silicone/PTFE septa (BGB®, Germany). Before extraction, each sample was preconditioned at 65 °C for 10 min agitating at 350 rpm, to release the volatiles to the headspace mimicking how the panellists smelled the samples. Volatiles in the headspace were extracted at 65 °C for 10 min without agitation and absorbed onto a Polydimethylsiloxane/Divinylbenzene/Carboxen 50/30 μm diameter, 1 cm length fibre (Supelco, PA, USA). After extraction, SPME fibres were desorbed onto the GC–MS by heating the fibre at 250 °C for 2 min. The GC–MS analysis settings were as previously described (Siccama, Pegiou, Eijkelboom, et al., 2021). A mixture of all samples was used for each quality control (QC) sample. QCs were analysed in the same way as all biological samples and were distributed along the analysis series. A range of n-alkanes (C_6 – C_{21}), prepared from a set of stock solutions of the individual alkanes, was analysed in the same way to calculate retention indices.

2.5.2. Analysis of non-volatile compounds

To profile the non-volatile compounds, ultra-performance LC–MS was performed. The semi-polar compounds were extracted by mixing 0.3 mL of each soup replicate with 0.9 mL 32.04 M methanol and 0.035 M formic acid followed by sonication and centrifugation, as described previously by De Vos et al. (2007). The LC–MS calibration and analysis settings were as previously described (Pegiou et al., 2021) using both negative and positive ionization modes of the Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermos Fisher Scientific™, Germany). A 0.3 mL from the same mixture of all samples as prepared and mentioned in 2.5.1, was used for each QC sample and analysed in the same way as the biological samples.

2.5.3. Untargeted metabolomics data processing workflow

All GC–MS and LC–MS data were processed following the untargeted metabolomics workflow centred around the software packages MetAlign and MSClust as described before (Pegiou et al., 2021). The obtained relative abundances of the reconstructed metabolites in the processed data were log-transformed and a correction for signal drift was carried

out, based on the QC samples (Wehrens et al., 2016).

2.5.4. Metabolite identification

Volatile metabolites were identified based on matching the reconstructed mass spectra and calculated RIs with authentic reference standards and those present in the NIST17 Mass Spectral Library and in-house databases. Non-volatile compounds were putatively identified based on matching their molecular ion mass and associated in-source fragments with the detected LC-MS asparagus compounds described by (Pegiou et al., 2021) and the online databases KnaPSAck (https://www.knapsackfamily.com/) and mzCloud (https://www.mzcloud.org/). The given level of identification (LOI) follows the guidelines of the Metabolomics Standards Initiative (Sumner et al., 2007).

2.6. Multivariate statistical analysis

The log-transformed metabolomics data were mean-centred, Pareto-scaled and variation between samples was initially explored by applying PCA. PCA analyses were performed using the R package ropls (Thevenot, Roux, Xu, Ezan, & Zunot, 2015). Random Forest was applied for the supervised analysis and variable selection, considering the composition of the data matrices obtained. The bootstrapping method, which is applied during random forest analyses, converges to the leave-one-out cross-validation method which is suitable for datasets with a limited number of observations, enabling a higher prediction performance. For the Random Forest analyses, log-transformed data were used and analyses were performed using the R package randomForest (Liaw & Wiener, 2002). The number of trees (ntree) and the number of variables (mtry) for each decision rule were optimized for the minimum prediction error for each model. The Random Forest classification approach was followed to determine those GC–MS and LC–MS compounds indicating the ingredient composition of a specific soup prototype. Two classification analyses were performed per dataset; one based on the primary asparagus component (3 classes: commercial, concentrate, spray-dried) and one based on the presence of asparagus fibre (2 classes: yes, no) (Table 1). The commercial powder was classified as fibre-containing as it is produced from whole asparagus pieces, thus, including the fibres. The performance of each model was assessed by the out-of-bag (OOB) error rate which corresponds to the prediction error after leave-one-out cross-validation. Variable importance was estimated by the mean decreased accuracy which expresses how much accuracy the model loses by excluding each variable. Compounds with Mean Decrease Accuracy > 1 were considered relevant. Hierarchical clustering (HCA) of the soups was subsequently performed focusing on the selected variables after log-transforming and autoscaling their abundances, and this was visualised in a heatmap. The HCA analysis and visualisation were performed using the R packages pheatmap and ggplot2 in RStudio with R version 4.1.1 (2021-08-10). The Random Forest regression approach was followed to determine which individual compounds have a predicting relevance to sensory attributes having a QI > 0.65 and p-adjusted < 0.05 (Table S2). The performance of each model was assessed by the mean square error (MSE). Variable importance was estimated by the increase in mean square error (%IncMSE) which expresses how much the percentage of prediction error increases by excluding each variable. Compounds with %IncMSE > 1.50 were considered relevant. Microsoft Excel and PowerPoint (version 2104, 2021) were used for additional data analysis and visualisation.

2.7. GC-Olfactometry-MS

The regression analyses highlighted 18 volatile compounds as being correlated to specific sensory attributes of which eight could be unambiguously identified (level 1). A mixture of the reference standards of these eight compounds was analysed with GC-O-MS, to determine whether these volatiles are odour-active within the concentration range detected in the soups without the extra addition of the flavour mix. A GC column splitter outlet was used to split 1:1 towards the MS and the olfactory detection port (ODP2, Gerstel, The Netherlands). The reference standards were dissolved in methanol and were used to prepare a solution in water comprising dimethyl sulphide (5 μg/mL), pentanal (5 μg/mL), 1-hexanol (5 μg/mL), 1,3-dimethylbenzene (0.2 μg/mL), 1-octen-3-ol (0.5 μg/mL), octanal (0.05 μg/mL), *(E)*-2-heptenal (0.2 μg/mL) and 2-methoxy-3-isopropyl pyrazine (0.2 μg/mL). The same SPME GC–MS conditions as described in section 2.5.1 were used except that the GC oven temperature program was adjusted to shorten total run time to 24 min by increasing the ramp to 20 °C/min after 15 min. For sniffing and assigning the individual peaks to a specific aroma attribute (if any), three assessors were recruited within the laboratory. Each assessor smelled the GC-O profile of the prepared standard solution and noted down the perceived aroma per compound at the given retention times without knowing which compound corresponds to which peak.

2.8. Physical characteristics

Selective physical properties of the dry powders and soup prototypes were analysed. The particle size distributions of the commercial asparagus powder, spray-dried powder and asparagus fibre were measured using a Mastersizer 3000 analyser (Malvern Inc, Malvern, UK) with the dry powder disperser Aero S. The size distributions of the commercial asparagus powder and asparagus fibre were analysed in the non-spherical analysis mode using the refractive index (RI) of cellulose, i.e. 1.468, since cellulose was considered the most abundant compound. The spray-dried powders were analysed with the spherical analysis mode using the RI of maltodextrin, i.e. 1.670. Furthermore, the particle size distributions of the soup prototypes were analysed in the Hydro MV module and the Mastersizer 3000 with the non-spherical analysis mode. The RI of cellulose (1.468) was used for the dispersed phase and 1.330 for the continuous phase (water). In addition, the particle size distribution of pure starch dissolved in hot water was measured using the spherical analysis mode and the RI of starch (1.450).

The viscosity of the soups was determined with the Anton Paar rheometer (MCR301, Anton Paar GmbH, Graz, Austria) with a concentric cylinder geometry (CC-27). A shear rate sweep with a logarithmic increasing shear rate from 1 to 1000 s^{-1} was performed. The rheology measurements were performed at 40 °C, which is assumed to be the relevant temperature inside the mouth before swallowing the soup (Deblais et al., 2021). The viscosities of the soups at 50 s^{-1} are reported (Table 1). This shear rate has been adopted as the oral shear rate standard by the National Dysphagia Diet task force and is considered a reasonable order of magnitude for swallowing liquids (Ong, Steele, & Duizer, 2018; Popa et al., 2013).

Microscopy images of the soups were taken using a light microscope (Carl Zeiss AxioScope, Jena, Germany) after the soup samples were vortexed to ensure homogeneity. A drop of the sample was placed on a microscopic slide under a coverslip. The images were captured (AxioCam MRc 5 camera) at a 20x magnification.

3. Results

3.1. The sensory profiles of the asparagus soups

Ten professional panellists evaluated the soup prototypes on 24 sensory attributes (Table S2). PCA was performed on the sensory profiles and > 79 % of the overall variance was explained by the first two principal components (Fig. 1A). The soup containing the commercial powder (C) had a similar sensory profile as the soups containing the concentrate or the spray-dried powder with fibre (JA and SA), as these are located close to each other on the PCA plot. These three prototypes had a contrasting sensory profile to the soups with the concentrate or the spray-dried powder without fibre (J and S), as well as the one with the flavour-supplemented commercial powder (CF), which, along PC2, was separated from all the other soups (Fig. 1A). The loadings (sensory

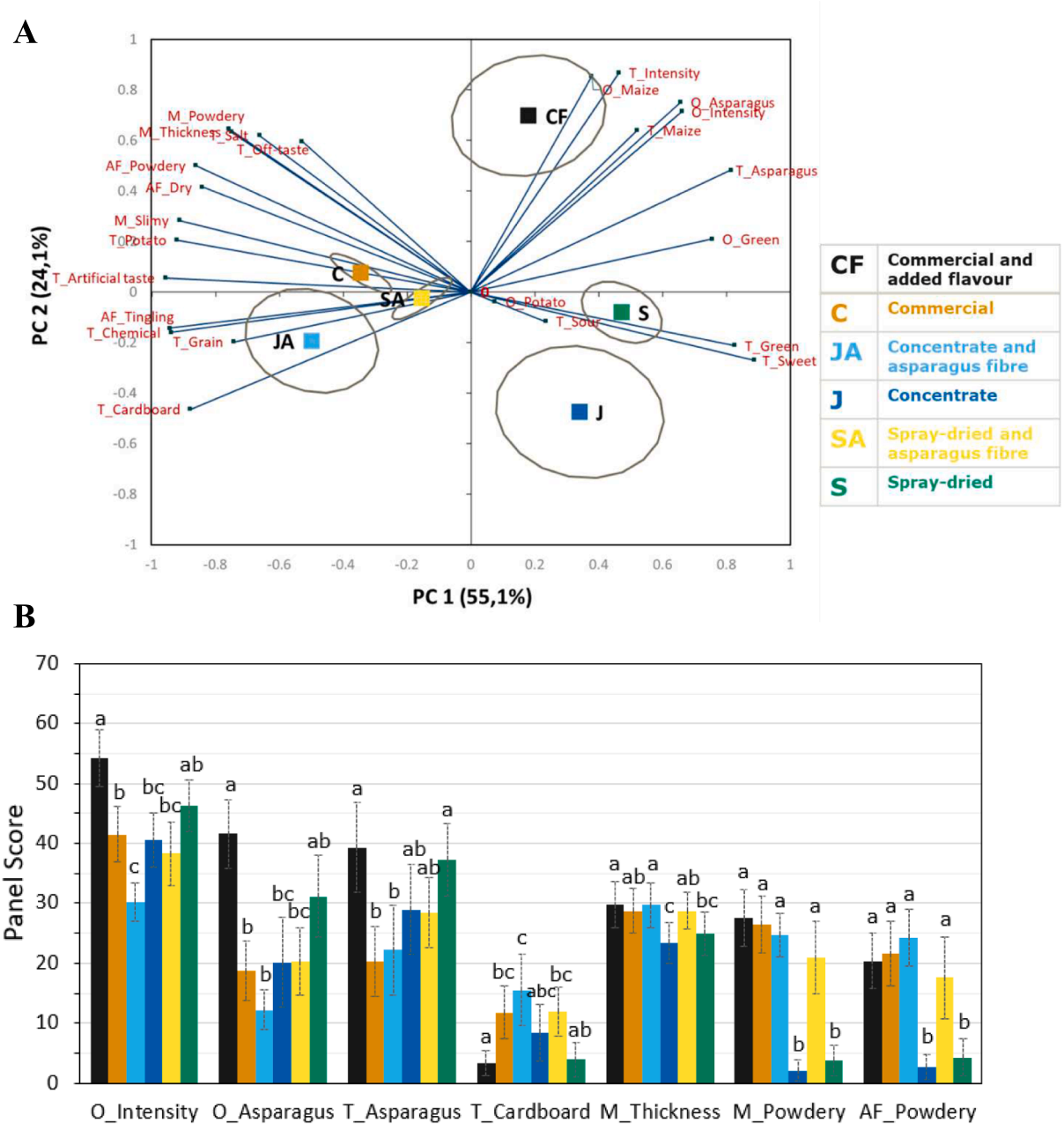

Fig. 1. Statistical analysis of the sensory evaluation data of the six soup prototypes from ten trained professional panellists. Score range: 0 – 100 with min = 0 and max = 82.7. (**A**): PCA biplot of the six different soups and the 24 sensory attributes that were scored during the evaluation which are labelled as in Table S2. Data points representing the soups are based on the average of all scores from all panellists and ellipses show the standard deviation. Data points are coloured based on the asparagus ingredients of the soups as presented in Table 1. The % of explained variance per PC is provided in parentheses on the x and y axes. (**B**): Bar graphs of the average score for seven selected sensory attributes that showed significant differences between the six soup prototypes related to odour (O), taste (T) mouthfeel (M) and after-feel (AF). The error bars show the 95 % confidence intervals based on the scores of 10 panellists who evaluated each prototype twice (n = 20). Bars are coloured based on the asparagus ingredients of the soups as in shown in (A). The letters indicate significant differences between the soup prototypes per attribute based on pairwise comparisons after two-way ANOVA. P-values were adjusted with the Benjamin Hochberg FDR procedure (significant p-value < 0.05).

attributes) being close to each other were positively correlated while attributes being located at opposite directions in Fig. 1A were negatively correlated. Likewise, soups located close to specific attributes obtained a high score for those sensory characteristics. For instance, the 'CF' soups scored high on the asparagus odour (O_Asparagus), asparagus taste (T_Asparagus) and the overall aroma intensity (O_Intensity) (Fig. 1A). These scores were significantly higher (p adjusted < 0.05) compared to all soups except for the 'S' prototype (Fig. 1B). In contrast, the soups containing the commercial powder (C) or the in-house processed ingredients with asparagus fibre (SA, JA) had a significantly higher score on the cardboard taste (T_Cardboard) compared to the other soups (Fig. 1A,B). Significant differences between the soup prototypes were also observed regarding the thickness (M_Thickness), powdery mouthfeel and after-feel (M_Powdery, AF_Powdery) (Fig. 1B, Table S2). The samples without asparagus fibre, i.e. 'J' and 'S', scored significantly lower on the powdery mouthfeel and after-feel compared to all the other soups. The 'J' prototype scored also significantly lower on thickness (Fig. 1B).

The sensory attributes that had a significantly different score between any of the soup prototypes (p adjusted < 0.05 and QI > 0.65)

(Table S2) were further investigated by combining the sensory with the metabolomics results as indicated below.

3.2. The metabolite profiles of the asparagus soups

The chemical composition of the soups was studied regarding the secondary metabolites using SPME GC–MS (volatiles) and LC-MS (non-volatiles). Following an untargeted metabolomics workflow for processing the raw data the compounds retained for further examination were 85 volatiles and 951 non-volatiles (both ionisation modes). Sulfur-containing compounds, furans, pyrazines, aldehydes, alcohols, ketones, aromatics, hydrocarbons, flavonoid glucosides and saponins were among the main groups of annotated compounds (Table S3, S4). All compounds had a lower relative standard deviation (RSD) across the QCs than the biological samples and the mean RSD across the QCs was lower than 0.25, indicating good technical reproducibility.

The GC–MS and LC-MS profiles were studied separately by PCA to explore the variation between the soup prototypes (Fig. 2A,B). The main differentiation was detected along PC1, explaining 41 % and 21 % of the overall variation of the volatiles and non-volatiles, respectively, and was between the soups containing the commercial powder and those containing the in-house processed ingredients (Fig. 2A,B). The metabolites with the highest contribution to the observed separation of the soups are depicted in Fig. 2C and 2D. These were one thiazole, acetoin, ethanol, one nitrogen-containing volatile compound (Fig. 2C) and three LC-MS compounds (pos_120, neg_334 and neg_309 in Fig. 2D) being detected at high levels in the in-house processed ingredients, and dimethyl disulphide (DMDS), *(E)*-2-heptenal, decanal, 2-butyl-2-octenal (Fig. 2C) and four LC-MS compounds (neg_71, neg_575, pos_521 and pos_727 in Fig. 2D) being detected at high levels in the commercial powder. Among the prototypes with the in-house ingredients, the presence of asparagus fibre appeared to influence the metabolite composition, which is most evident from the non-volatiles (Fig. 2B). The impact of adding the flavour mix to the commercial powder is only reflected by the volatiles (Fig. 2A). The three volatiles that were highly abundant in the flavour-supplemented soups were dimethyl sulphide (DMS), 2-methoxy-3-isopropylpyarzine and 2-methoxy-3-isobutylpyrazine (Fig. 2C).

3.3. Compounds specific for the asparagus ingredients of the instant soups

The metabolite profiles of the soup prototypes varied based on the asparagus ingredients. The addition of asparagus fibre to the in-house prototypes appeared to have a dominant effect on their metabolite profiles (Fig. 2A,B). The composition of the soups was therefore, examined by performing two Random Forest classification analyses to highlight the discriminatory compounds. One analysis for classifying the soup prototypes based on the primary asparagus component (commercial, concentrate, spray-dried) and one based on the presence of asparagus fibre (CF, C, JA, SA contain fibre) (Table 1).

The Random Forest classification models highlighted eight compounds which were indicative for the primary asparagus component (mean OOB error rate from the two metabolomics datasets 26.7 %) and 12 compounds indicating the presence (six compounds) or absence (six compounds) of asparagus fibre (mean OOB error rate from the two

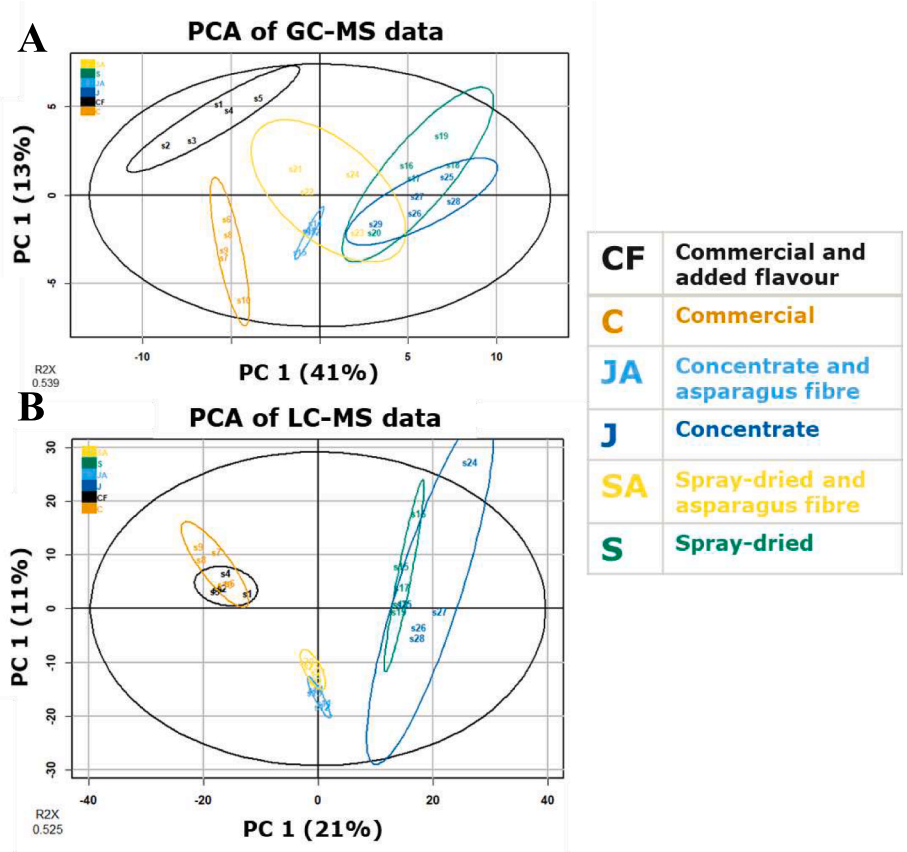

Fig. 2. Principal component analyses (PCA) of the metabolite profiles of the six soup prototypes. PCA score plots based on (A) 85 volatiles and (B) 951 non-volatiles. The first two PCs are presented, and the explained variance is shown in parentheses on the axes. The black ellipses represent the 95 % confidence interval from the Hotelling T2 function. Data points are coloured based on the asparagus ingredients of each soup as presented in Table 1. The coloured ellipses show the confidence interval for each soup prototype based on the analysed replicates (n = 4 or 5).. (C): PCA loading plot of the GC–MS profile with the top12 variables highlighted. Annotation is given in case of LOI < 3. (D): PCA loading plot of the LC-MS profiles with highlighted the variables having high contribution. Analyses and plots were made using the ropls R package.

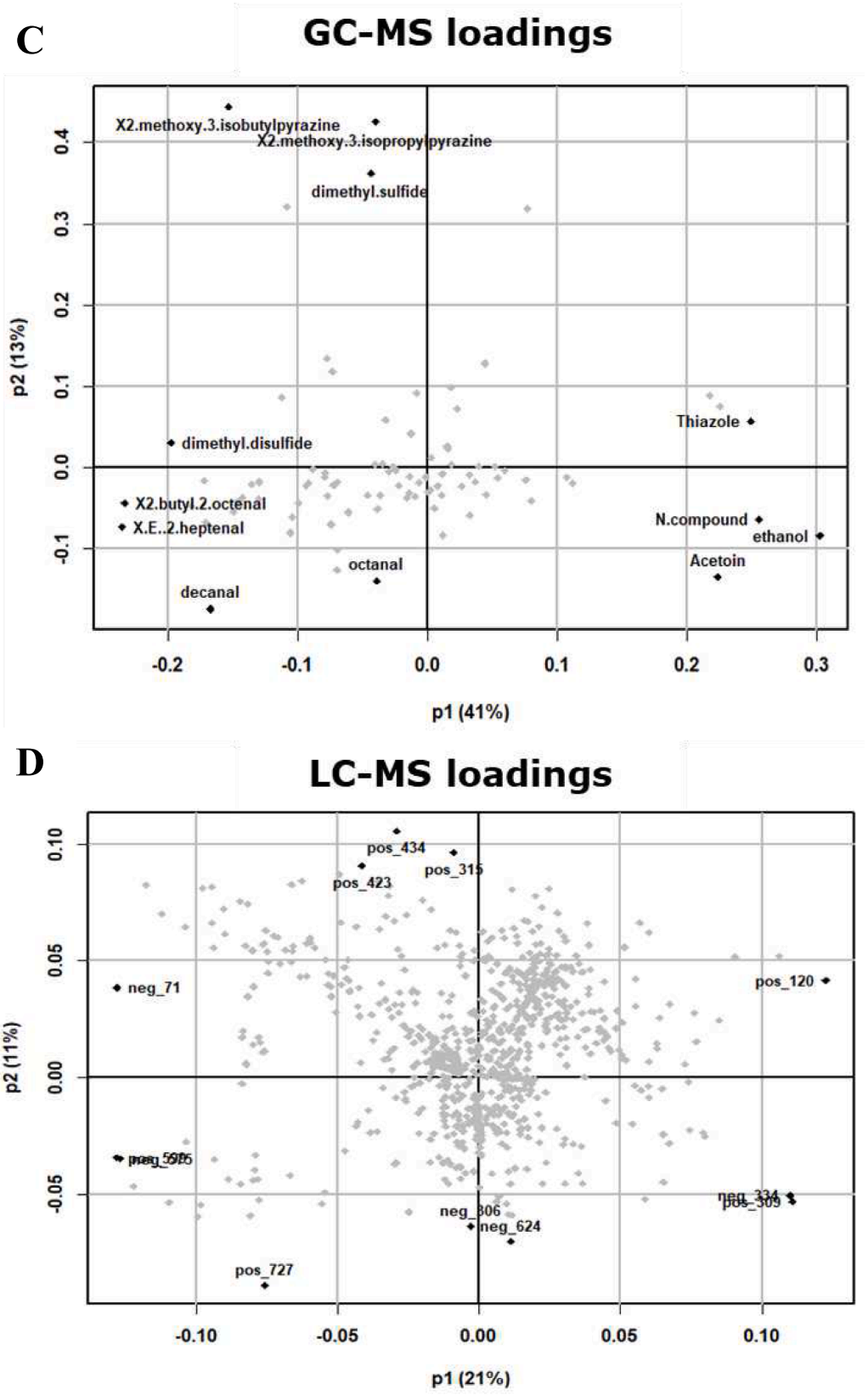

Fig. 2. (*continued*).

metabolomics datasets 3.6 %). HCA of the soups based on the 20 discriminatory compounds demonstrated the grouping of the prototypes according to the asparagus ingredients (Fig. 3A). The soups without asparagus fibre are in cluster I and the soups with asparagus fibre are in cluster II (Fig. 3A). The 20 compounds were clustered in three main groups (Fig. 3A). The compounds in groups 1 and 2 are indicative of the

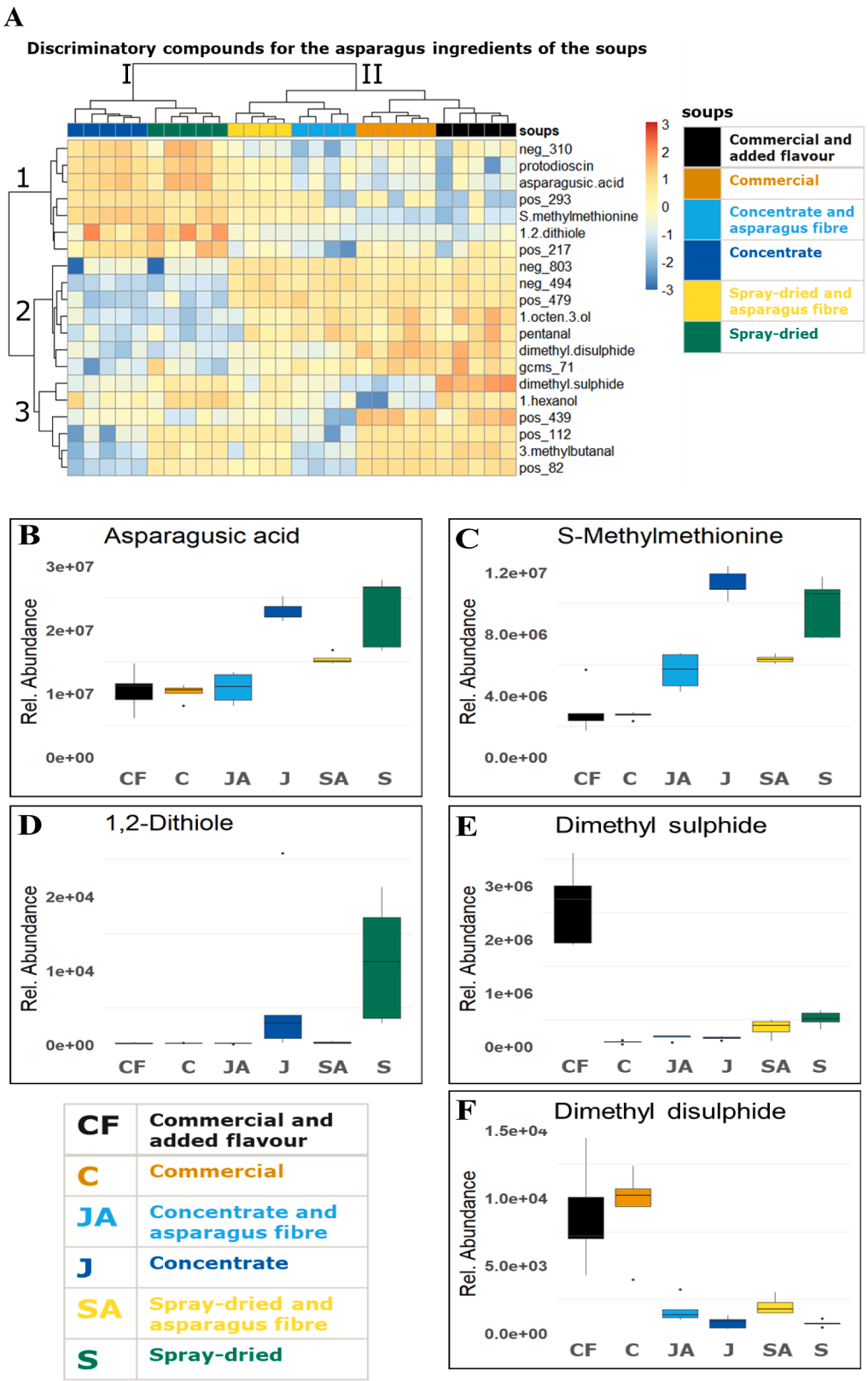

Fig. 3. Visualisation and inspection of compounds found to be specific for the composition of the asparagus soups based on the Random Forest classification analyses. (**A**) Heatmap of the 20 highlighted compounds (Mean Decreased Accuracy > 1) and their abundances in the soup prototypes (Fibre-specific: protodioscin, pos_479, asparagusic acid, neg_494, neg_803, neg_310, S-methylmethionine, pos_217, gcms_71, dimethyl disulphide, 1,2-dithiole, pentanal. Ingredient-specific: pos_112, pos_439, pos_82, pos_293, 1-hexanol, 3-methylbutanal, 1-octen-3-ol, dimethyl sulphide). Data were log-transformed and scaled. Dendrograms correspond to the clustering of the soups or the compounds based on HCA using Ward's method. (**B – F**): The abundances of the five sulfur compounds shown in (A) in the six soup prototypes are presented as boxplots.

absence or presence of asparagus fibre in the soups, respectively. The compounds in group 3 were highly abundant in the dried powders (spray-dried and commercial), except for DMS and 1-hexanol, which were specifically more abundant in the spray-dried and the flavour-supplemented commercial powder (Fig. 3A). The five sulfur-containing metabolites were examined in detail considering their reported high relevance to asparagus flavour notes. Asparagusic acid, S-methylmethionine and 1,2-dithiole followed similar patterns and were more abundant in the prototypes without fibre (Fig. 3B – 3D). DMS was detected at high levels in the 'CF', but also in the 'S' soups (Fig. 3E) and DMDS was highly abundant in the 'C' and 'CF' soups (Fig. 3F).

3.4. Compounds relevant for the perception of asparagus flavour notes

To retrieve compounds that are of specific relevance to certain sensory attributes, the outcomes of the descriptive sensory analysis and the metabolomics evaluation of the asparagus soups, were combined using advanced statistical tools.

Of the 24 scored sensory attributes, 13 had significantly different scores between the soups (p adjusted < 0.05 and QI > 0.65) (Table S2). Random Forest regression analysis was performed to determine which metabolites potentially contribute to certain flavour characteristics. Five sensory attributes were reliably predicted by GC–MS profiles and three sensory attributes were reliably predicted by LC-MS profiles (MSE < 25). In addition, the Pearson's correlation coefficient between each compound and the linked sensory attribute was calculated to help in the interpretation of their relationship (Tables 2A and 2B).

Concerning the GC–MS profiles, in total 18 volatiles were highlighted as significantly correlating to asparagus odour, overall odour and taste intensity and the two powdery attributes (Table 2A). The highlighted relationships between volatiles and sensory attributes were mainly positively correlated. Specifically, the volatiles that were found to significantly correlate to high perception of asparagus odour and taste were DMS, 2-methoxy-3-isopropylpyrazine, 3-methyl-1-butanol, 1,3-dimethylbenzene and one unidentified compound (Table 2A).

Concerning the LC-MS profiles, six compounds were found to be highly associated with cardboard taste and 12 compounds were linked to the two powdery attributes (Table 2B). For each compound, the provided monoisotopic mass was calculated based on the base peak and the Pearson's correlation coefficient indicates whether the presence (positively correlated) or absence (negatively correlated) of these specific compounds can predict the perception of the linked flavour attribute. For example, S-adenosylhomocysteine was positively correlated with the powdery mouthfeel while protodioscin was negatively correlated with the powdery experience (Table 2B).

3.5. GC-Olfactory-MS confirms the sensory-activity of asparagus volatiles

To test whether the volatile compounds that were associated with specific flavour attributes are odour-active a series of GC-O-MS analyses were executed. A mix of eight identified volatile compounds that were purchased as pure standards was prepared. The mix was then analysed with GC-O-MS and smelled by three assessors in independent runs. Retention times of the eight compounds were such that each peak could be readily evaluated separately. Results showed that 2-methoxy-3-isopropyl pyrazine, 1-octen-3-ol and *(E)*-2-heptenal were odour-active based on all three assessors who also recorded similar aromas (Table 2A). DMS, pentanal, 1-hexanol and octanal were also odour-active and could be detected by two of the three assessors, while 1,3-dimethylbenzene was not perceived by any of the assessors (Table 2A). Interestingly, when the three assessors were finally asked to smell the mixture of the standards from the GC vial, two directly described this as being like asparagus.

3.6. Physical properties and mouthfeel of the soup prototypes

The particle size distributions of the dry asparagus ingredients (i.e. commercial, spray-dried powder and asparagus fibre) were analysed using the dry powder dispenser and different size distributions were observed (Fig. S1A). Despite the fine milling and sieving, the asparagus fibre preparation consisted of the largest particles, with a volume mean particle diameter (D[4,3]) of 236 μm, whereas the commercial and the spray-dried powders were characterised with a D[4,3] of 160 and 155 μm, respectively. Therefore, it was anticipated that the presence of fibre

Table 2A
List of the volatile compounds that were linked to a sensory attribute based on Random Forest regression (%IncMSE > 1.50). The Pearson's correlation coefficient of each compound with the related sensory attribute was calculated to interpret the relationship between them. The aroma characteristics per compound based on the Good Scents and FooDB databases are provided. In the final column, the aroma characteristics per compound as perceived by the assessors during the GC-O-MS analysis are listed.

Compound / ID	Sensory attribute(s)	Pearson's correlation coefficient	Aroma attribute based on databases[1]	Aroma attribute perceived at the ODP[2]
Dimethyl sulphide	O_Intensity	0.55	onion, cabbage	earthy, asparagus, potato
	O_Asparagus	0.59		
2-Methoxy-3-isopropylpyrazine	O_Intensity	0.49	earthy, bean, pea	earthy, vegetable, asparagus
	O_Asparagus	0.52		
gcms_112	O_Intensity	0.20		Na
1-Hexanol	O_Intensity	0.44	herbal, fruity	herbal, grassy
2-Methoxy-3-isobutylpyrazine	O_Intensity	0.36	bell pepper, earthy	Na
gcms_115	O_Asparagus	0.30		Na
3-Methyl-1-butanol	O_Asparagus	0.43	fruity, banana	Na
1,3-Dimethylbenzene	O_Asparagus	0.01	–	not detectable
gcms_105	T_Intensity	0.30		Na
Octanal	T_Intensity	0.20	waxy, orange peel	fruity, floral
gcms_66	T_Intensity	0.52		Na
Pentanal	M_powdery	0.84	fermented, nutty	sweet, floral
	AF_Powdery	0.57		
1-Octen-3-ol	M_Powdery	0.80	earthy, mushroom	earthy, mushroom
	AF_Powdery	0.47		
gcms_71 (alkane)	M_Powdery	0.65		Na
	AF_Powdery	0.38		
gcms_43 (nitrogen-containing)	M_Powdery	−0.78		Na
(E)-2-Heptenal	M_Powdery	0.72	fatty, pungent, intense	strong chemical
n-Caproic acid vinyl ester	M_Powdery	0.66	–	Na
gcms_76 (sulfur-containing)	M_Powdery	−0.77		Na

[1] aroma attribute based on The Good Scents (https://www.thegoodscentscompany.com/) and FooDB (https://foodb.ca/) databases.
[2] aroma attribute perceived at the ODP by at least two of the three assessors. Na when no reference standard was analysed.

Table 2B
List of the non-volatile compounds that were linked to a sensory attribute based on Random Forest regression (%IncMSE > 1.50). The Pearson's correlation coefficient of each compound with the specific attribute was calculated to interpret the relationship between them. The calculated monoisotopic mass of each compound is provided. For the compound ID pos/neg refers to the mode of detection used.

Compound / ID	Sensory Attribute	Pearson's correlation coefficient	Calculated monoisotopic mass
neg_204	T_Cardboard	−0.45	312.0337
neg_411	T_Cardboard	−0.33	505.2473
neg_97	T_Cardboard	0.24	328.9810
pos_315	T_Cardboard	−0.52	355.0995
pos_479	T_Cardboard	0.51	168.0798
pos_490	T_Cardboard	−0.18	171.8606
Asparagusic acid	M_Powdery	−0.85	149.9806
S-Adenosylhomocysteine	M_Powdery	0.79	384.1209
neg_496	M_Powdery	−0.08	337.8662
neg_601	M_Powdery	0.62	312.2301
neg_629	M_Powdery	0.66	364.3021
neg_768	M_Powdery	0.78	998.5092
pos_371	M_Powdery	−0.83	1065.5092
neg_667	AF_Powdery	0.19	335.8705
Protodioscin	AF_Powdery	−0.66	1048.5468
pos_150	AF_Powdery	−0.61	304.0675
pos_283	AF_Powdery	−0.61	364.9045
pos_290	AF_Powdery	−0.57	422.1227

might negatively influence the sensory perception of the soups, giving a sandy and thicker mouthfeel. Hence, physical properties of the soup samples were measured and then related to the perceived mouthfeel. Despite the differences in perceived sensorial properties (i.e. powdery mouthfeel) (Fig. 1B), the D[4,3] values indicated no significant differences among the soup prototypes (Table 1). The particle size distributions of the soups were almost the same despite the different asparagus ingredients used, which were also identical to the particle size distribution of pure starch suspended in hot water (Fig. S1B). The small quantity of asparagus fibre present did not yield changes in the particle size distribution of the soups. In addition, the viscosity of soups measured at 50 s^{-1} shear rate and 40°C did not differ significantly, and all soup samples showed similar shear-thinning behaviour (data not shown).

The soup prototypes containing spray-dried powder with or without asparagus fibre were also observed under the light microscope (Fig. S2). As expected, the presence of asparagus fibre was clearly visible in the 'SA' soup as a dense and elongated particle. The other particles that were visualised in both 'S' and 'SA' soups were starch granules given their morphology of swollen and round particles. Other soup prototypes were also analysed and the images of the samples with fibre (CF, C and JA) were similar to the 'SA' soup, while the sample without fibre (J) was similar to the image of 'S' (data not shown).

4. Discussion

4.1. A positive impact of spray drying on the flavour of instant asparagus soups

The flavour-supplemented commercial powder scored significantly higher than the commercial powder on its own with regards to asparagus flavour and flavour intensity (Fig. 1A,B), indicating the effectivity of the added flavour mix on the overall sensory perception. However, the ultimate goal for industry would be to design a product which can avoid this required addition of flavour supplements in food products (Román et al., 2017). This would result in a more natural and sustainable solution.

The sensory and the metabolomics results indicate that the soup prototypes with split-stream processed asparagus ingredients but without asparagus fibre had similarities with the flavour-supplemented commercial powder (Fig. 1A, 2A,B). Specifically, the soups containing the spray-dried powder were awarded higher scores on the key attributes asparagus odour and taste than the soups containing the commercial powder without the supplementary flavour mix (Fig. 1B). This indicated that the sensory profile of the spray-dried powder was richer in asparagus flavours compared to the hot-air dried commercial powder. The soups containing the spray-dried powder had slightly higher scores on the asparagus odour and taste attributes than those containing the concentrate (Fig. 1B), highlighting a positive impact of spray drying on the flavour profile. This confirms the richer flavour of the spray-dried powder compared to the concentrate as was also suggested based on the chemical composition of the processed asparagus ingredients (Siccama, Pegiou, Zhang, et al., 2021).

Known asparagus aroma compounds, such as DMS and 2-methoxy-3-isopropylpyrazine (Hoberg et al., 2003; Tressl, Holzer, & Apetz, 1977b; Ulrich et al., 2001) were seen to be highly abundant in the flavour-supplemented commercial powder (Fig. 2C, Table S3) and were also positively correlated with the asparagus flavour attributes (Table 2A). However, when comparing the spray-dried to the commercial powder, these key odorants were more abundant in the spray-dried powder and hardly detected in the commercial one (Fig. 3E, Table S3). This confirms the successful retention of key aroma compounds upon spray-drying, as was predicted previously (Siccama, Pegiou, Zhang, et al., 2021).

With respect to the sulfur-containing flavour compounds, which have been previously investigated in cooked asparagus spears highlighting their sensory-relevance (Tressl et al., 1977b; Ulrich et al., 2001), we detected and monitored two non-volatile precursors (S-methylmethionine and asparagusic acid) and the volatiles DMS, DMDS and 1,2-dithiole (Fig. 3B-F). The non-volatile precursors which can be converted on heating to DMS and DMDS were more abundant in the spray-dried powder compared to the commercial one (Fig. 3B,C). This explains the higher levels of DMS in the spray-dried powder compared to the pure commercial powder (Fig. 3E). Therefore, spray drying which we predict leads to the encapsulation of aroma compounds (Siccama, Pegiou, Zhang, et al., 2021) may also have a positive impact on retaining key flavour precursors. The fact that DMDS is equally high in both prototypes with the commercial powder indicates that it was already present in the actual commercial powder (and not in the flavour mix). Given that DMDS is also formed during heat treatment (Belitz, Grosch, & Schieberle, 2008; Parry, Mizusawa, Chiu, Naidu, & Ricciardone, 1985; Tressl, Holzer, & Apetz, 1977a), the much higher abundance in the commercial powders compared to the others can be explained by the fact that the commercial powder has undergone a longer heat treatment than the in-house produced powders.

4.2. The negative impact of asparagus fibre on the flavour and mouthfeel of instant asparagus soups

During the split-stream processing of asparagus waste streams, as a first step, the fibres are separated from the juice (Siccama, Pegiou, Eijkelboom, et al., 2021). To fully exploit the generated asparagus "waste" in a sustainable and circular manner, it was considered that the asparagus fibre could potentially be reintroduced into the production process to make powders. It was hypothesized, that the addition of the processed fibre in the final soup formulation could (positively) affect the mouthfeel sensation by providing more structure and thickness to the soups, thereby having a higher sensory impact. Lyly et al. (2004) evaluated the sensory characteristics and rheological properties of vegetable soups enriched with soluble fibres in the form of oat and barley β-glucans. They found that the concentration of β-glucan positively correlated to the viscosity and perceived thickness of the soups. Alqahtani et al. (2014) investigated the addition of insoluble fibre (i.e. orange, wheat and oat fibre) to beverages and demonstrated that the fibre-enriched beverages are perceived favourably by the panellists. Furthermore, the oat fibre samples received the highest score on overall acceptability, even higher than the commercial prototype. In the study presented here, samples with added asparagus fibres scored higher on cardboard taste, artificial taste, off-taste and powdery mouthfeel (Fig. 1A), which may suggest that the addition of fibres negatively impacts the overall sensory perception.

Interestingly, despite these differences in mouthfeel as perceived by the panellists (Fig. 1B), the volume mean particle diameter (D[4,3]) and viscosity of the soups were not significantly different (Table 1). While examining the morphology of the soup prototypes under a light microscope, we saw clear differences between samples with and without fibre. The asparagus fibre was different in shape compared to the starch granules (Fig. S2). The textural perception of particles is strongly influenced by the size, shape and deformability of the particles (Appelqvist, Cochet-Broch, Poelman, & Day, 2015; Tyle, 1993). Although the starch granules and asparagus fibres were found to be in the same size range, the asparagus fibres were rigid insoluble particles that were less deformable compared to starch granules. In addition, asparagus fibres were more irregular in shape, due to the milling process. Tyle (1993) demonstrated that hard and angular particles result in more perceived grittiness than soft and round particles. Therefore, in this study, the perceived thicker and powdery mouthfeel of the soups containing asparagus fibre was possibly caused by the presence of these rigid and angular fibre particles.

The metabolite profiles of the asparagus instant soups which were affected by the presence of fibre (Fig. 2A,B, 3A) were also linked to the sensory data. Some of the fibre-specific metabolites (Fig. 3A) were also found to correlate with specific flavour attributes (Tables 2A,B). The compound pos_479 which was highly abundant in all the fibre-containing prototypes (Fig. 3A), was also highly positively correlated with the cardboard taste of the soups (Table 2B), suggesting that this non-volatile compound should be further investigated as an off-flavour in the asparagus soups (Whitson, Miracle, & Drake, 2010). The volatile compounds, pentanal, 1-octen-3-ol and gcms_71 which were highly abundant in the fibre-containing prototypes (Fig. 3A), were positively correlated with the powdery mouthfeel (Table 2A). The powdery sensation for an instant soup is likely undesirable (Ssepuuya, Katongole, & Tumuhimbise, 2018), as it was also indicated by the sensory evaluation of the soups where the powdery attributes were positively correlated with the off-taste and artificial attributes (Fig. 1A,B). The fibre-containing soups scored higher on powdery mouthfeel compared to the other soups (Fig. 1A,B), suggesting a negative impact of the asparagus fibre on the flavour sensation. Thus, the fibre-specific metabolites may potentially be related to off-flavours if they were sensory-active.

4.3. Proposal of new asparagus odorants and off-flavours

The previously reported key asparagus odorants DMS, 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine (Ulrich et al., 2001) were in this study strongly correlated with important asparagus flavour attributes of the soups (Table 2A) and moreover DMS and 2-methoxy-3-isopropyl pyrazine were also perceived to have an earthy and asparagus smell by at least two of the three assessors during the GC-O-MS study (Table 2A). Additionally, 3-methyl-1-butanol, 1-hexanol, octanal and four unidentified compounds (gcms_66, gcms_105, gcms_112 and gcms_115) were also highly associated with sensory attributes, specifically with asparagus odour, overall odour and taste intensity. The three annotated compounds have fruity and herbal aroma attributes (Table 2A) that resemble fresh asparagus. The aromatic compound 1,3-dimethylbenzene was linked to asparagus odour, but in the GC-O-MS analysis, it was not detected by any of the assessors (Table 2A). This is in agreement with the Good Scents database (https://www.thegoodscentscompany.com/) which does not associate it with a detectable aroma. It may be that this compound acts as an aroma enhancer when present in a matrix but this would require additional studies using an aroma matrix dilution series.

The powdery sensation which was evaluated regarding the mouthfeel and after-feel (Table S2) is not a preferred feature for soup products (Ssepuuya et al., 2018) and this was confirmed as the powdery attributes were positively correlated with off-taste (Fig. 1A), although potential causality should be further investigated for their contribution to off-flavours. Asparagusic acid, two saponins (protodioscin and pos_371) (Table 2B) and two volatiles (one nitrogen-containing and one sulfur-containing compound) (Table 2A) were negatively correlated with the powdery mouthfeel implying their contribution to "positive" asparagus flavours. The essential role of asparagusic acid in the formation of important sulfur-containing asparagus odorants is known (Parry et al., 1985; Tressl et al., 1977b). Further investigation of the two volatiles (gcms_76 and gcms_43), which were highly abundant in the prototype soups without fibre (Table S3) might lead to the discovery of two new asparagus odorants. In contrast, pentanal, *(E)*-2-heptenal, 1-octen-3-ol and *n*-caproic acid vinyl ester which were highly positively correlated with the powdery sensation of the soups are potential off-flavours worthy of attention. Blanda, Cerretani, Comandini, Toschi, and Lercker (2010) investigated the volatile composition of boiled potatoes and the influence of additives on the flavour. They showed that increased levels of medium-chained aldehydes (e.g. pentanal, 2-heptenal) led to the formation of off-flavours as perceived by the panellists. Pentanal, *(E)*-2-heptenal and 1-octen-3-ol have been previously reported as key odorants in cooked white asparagus (Ulrich et al., 2001) but here are suggested as causal off-flavours. This contradiction is likely related to the different matrix of the asparagus materials used (cooked spear versus instant soup) as well as the concentration of the specific compounds and the rest of the profile. Regarding the non-volatile profile of the asparagus soups, six compounds were positively correlated with the cardboard taste attribute (Table 2B), suggesting that these compounds are also potentially causal off-flavours. Table 3 summarizes the outcomes of our study and our proposal with respect to the asparagus flavours and off-flavours.

5. Conclusion

This sensory descriptive analysis showed that soup prototypes prepared with spray-dried asparagus powder made from concentrated asparagus juice had similar flavour notes to those prepared from a flavour-supplemented commercial powder. Adding asparagus fibre negatively affected the flavour and mouthfeel of the soup prototypes. Using advanced metabolomics tools, a chemical characterization of the soups was performed and the datasets of the two analyses (sensory and metabolomics) were fused and examined following Random Forest approaches. Not only key known asparagus odorants were highlighted and

Table 3
Summarizing list of the confirmed asparagus sensory-relevant compounds which are known from the literature and the compounds that are proposed as new (off-) flavours based on this study. For the unidentified non-volatiles pos/neg refers to the mode of detection used.

Volatiles	Non-volatiles
Known flavour compounds (confirmed)	
Dimethyl sulphide	Asparagusic
2-Methoxy-3-isopropyl pyrazine	acid
	Protodioscin
New flavour compounds (suggested)	
3-Methyl-1-butanol1-HexanolOctanalgcms_112 (alkane)	
gcms_115 (ether)gcms_43	
(*N*-containing compound)gcms_76 (S-containing compound)	
Causal off-flavours (suggested)	
Pentanal	neg_204
(E)-2-Heptenal1-Octen-3-ol	neg_411
n-Caproic acid vinyl ester	neg_97
	pos_315
	pos_479
	pos_490

confirmed, but we also suggest new compounds with potential relevance for the sensory profile of the asparagus ingredients. In conclusion, this study has revealed that spray drying of asparagus concentrate is a promising processing method to produce flavour-rich asparagus powder, as compared to the conventional oven-drying process. A similar process may be tested to upcycle other vegetable waste streams to produce flavour-rich food ingredients, in turn contributing to the sustainability of food systems. Ultimately, this could reduce the usage of supplemental flavourings in food products to make them more natural.

CRediT authorship contribution statement

Eirini Pegiou: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Joanne W. Siccama:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Roland Mumm:** Supervision, Methodology, Writing – review & editing. **Lu Zhang:** Supervision, Methodology, Writing – review & editing. **Doris M. Jacobs:** Methodology, Resources. **Xavier Y. Lauteslager:** Methodology, Resources. **Marcia T. Knoop:** Methodology, Resources. **Maarten A.I. Schutyser:** Conceptualization, Supervision, Project administration, Writing – review & editing. **Robert D. Hall:** Conceptualization, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Doris M. Jacobs, Xavier Y. Lauteslager and Marcia Knoop are employees of Unilever, which manufactures and markets consumer food products, including asparagus soups. All other authors declare no potential conflict of interest related to the presented work.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134986.

References

Alqahtani, N. K., Ashton, J., Katopo, L., Haque, E., Jones, O. A. H., & Kasapis, S. (2014). Consistency of UHT beverages enriched with insoluble fibre during storage. *Bioactive Carbohydrates and Dietary Fibre, 4*(1), 84–92. https://doi.org/10.1016/J.BCDF.2014.06.005

Appelqvist, I. A. M., Cochet-Broch, M., Poelman, A. A. M., & Day, L. (2015). Morphologies, volume fraction and viscosity of cell wall particle dispersions particle related to sensory perception. *Food Hydrocolloids, 44*, 198–207. https://doi.org/10.1016/J.FOODHYD.2014.09.012

Belitz, H. D., Grosch, W., & Schieberle, P. (2008). Food Chemistry. *Springer Science & Business Media.* https://books.google.nl/books?id=hftDAAAAQBAJ&lr=&source=gbs_navlinks_s.

Bettenhausen, H. M., Benson, A., Fisk, S., Herb, D., Hernandez, J., Lim, J., … Hayes, P. M. (2020). Variation in Sensory Attributes and Volatile Compounds in Beers Brewed from Genetically Distinct Malts: An Integrated Sensory and Non-Targeted Metabolomics Approach. *Journal of the American Society of Brewing Chemists, 78*(2), 136–152. https://doi.org/10.1080/03610470.2019.1706037

Blanda, G., Cerretani, L., Comandini, P., Toschi, T. G., & Lercker, G. (2010). Investigation of off-odour and off-flavour development in boiled potatoes. *Food Chemistry, 118*(2), 283–290. https://doi.org/10.1016/j.foodchem.2009.04.135

Buvé, C., Neckebroeck, B., Haenen, A., Kebede, B., Hendrickx, M., Grauwet, T., & Van Loey, A. (2018). Combining untargeted, targeted and sensory data to investigate the impact of storage on food volatiles: A case study on strawberry juice. *Food Research International, 113*(July), 382–391. https://doi.org/10.1016/j.foodres.2018.07.022

Cebrián, G., Condón, S., & Mañas, P. (2017). Physiology of the inactivation of vegetative bacteria by thermal treatments: Mode of action, influence of environmental factors and inactivation kinetics. *Foods, 6*(12), 1–21. https://doi.org/10.3390/foods6120107

Davarzani, N., Diez-Simon, C., Großmann, J. L., Jacobs, D. M., van Doorn, R., van den Berg, M. A., … Westerhuis, J. A. (2021). Systematic selection of competing metabolomics methods in a metabolite-sensory relationship study. *Metabolomics, 17*(9), 1–12. https://doi.org/10.1007/s11306-021-01821-3

Dawid, C., & Hofmann, T. (2012). Identification of sensory-active phytochemicals in asparagus (Asparagus officinalis L.). *Journal of Agricultural and Food Chemistry, 60*(48), 11877–11888. https://doi.org/10.1021/jf3040868

De Vos, R. C. H., Moco, S., Lommen, A., Keurentjes, J. J. B., Bino, R. J., & Hall, R. D. (2007). Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nature Protocols, 2*(4), 778–791. https://doi.org/10.1038/nprot.2007.95

Deblais, A., den Hollander, E., Boucon, C., Blok, A. E., Veltkamp, B., Voudouris, P., … Velikov, K. P. (2021). Predicting thickness perception of liquid food products from their non-Newtonian rheology. *Nature Communications, 12*(1), 1–7. https://doi.org/10.1038/s41467-021-26687-w

Diez-Simon, C., Eichelsheim, C., Jacobs, D. M., Mumm, R., & Hall, R. D. (2021). Stir bar sorptive extraction of aroma compounds in soy sauce: Revealing the chemical diversity. *Food Research International, 144*(February), Article 110348. https://doi.org/10.1016/j.foodres.2021.110348

Greño, M., Plaza, M., Luisa Marina, M., & Castro Puyana, M. (2023). Untargeted HPLC-MS-based metabolomics approach to reveal cocoa powder adulterations. *Food Chemistry, 402*(September 2022). https://doi.org/10.1016/j.foodchem.2022.134209

Hoberg, E., Ulrich, D., Gottwald, J., & Rosen, A. (2003). Environmental influences on the sensory quality of asparagus officinalis l. *Acta Horticulturae, 604*, 395–401. https://doi.org/10.17660/ActaHortic.2003.604.42

Hyldig, G., & Green-Petersen, D. M. B. (2004). Quality index method-an objective tool for determination of sensory quality. *Journal of Aquatic Food Product Technology, 13*(4), 71–80. https://doi.org/10.1300/J030v13n04_06

Jacobs, D. M., van den Berg, M. A., & Hall, R. D. (2021). Towards superior plant-based foods using metabolomics. *Current Opinion in Biotechnology, 70*, 23–28. https://doi.org/10.1016/j.copbio.2020.08.010

Kamiloglu, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., Hall, R. D., & Capanoglu, E. (2016). *A Review on the Effect of Drying on Antioxidant Potential of Fruits and Vegetables*. doi:10.1080/10408398.2015.1045969.

Karam, M. C., Petit, J., Zimmer, D., Baudelaire Djantou, E., & Scher, J. (2016). Effects of drying and grinding in production of fruit and vegetable powders: A review. *Journal of Food Engineering, 188*, 32–49. https://doi.org/10.1016/j.jfoodeng.2016.05.001

Liaw, A., & Wiener, M. (2002). Classification and Regression by randomForest. *R News, 2*(3), 18–22.

Lyly, M., Salmenkallio-Marttila, M., Suortti, T., Autio, K., Poutanen, K., & Lähteenmäki, L. (2004). The sensory characteristics and rheological properties of soups containing oat and barley β-glucan before and after freezing. *LWT - Food Science and Technology, 37*(7), 749–761. https://doi.org/10.1016/J.LWT.2004.02.009

Nijhuis, H., Torringa, H., Muresan, S., Yuksel, D., Leguijt, C., & Kloek, W. (1998). Approaches to improving the quality of dried fruit and vegetables. *Trends in Food Science & Technology, 9*(1), 13–20. https://doi.org/10.1016/S0924-2244(97)00007-1

Ong, J. J. X., Steele, C. M., & Duizer, L. M. (2018). Challenges to assumptions regarding oral shear rate during oral processing and swallowing based on sensory testing with thickened liquids. *Food Hydrocolloids, 84*, 173–180. https://doi.org/10.1016/J.FOODHYD.2018.05.043

Parry, R. J., Mizusawa, A. E., Chiu, I. C., Naidu, M. V., & Ricciardone, M. (1985). Biosynthesis of sulfur compounds. Investigations of the biosynthesis of asparagusic acid. *Journal of the American Chemical Society, 107*(8), 2512–2521. https://doi.org/10.1021/ja00294a051

Pegiou, E., Mumm, R., Acharya, P., de Vos, R. C. H., & Hall, R. D. (2020). Green and white asparagus (Asparagus officinalis): A source of developmental, chemical and urinary intrigue. *Metabolites, 10*(1). https://doi.org/10.3390/metabo10010017

Pegiou, E., Zhu, Q., Pegios, P., De Vos, R. C. H., Mumm, R., Hall, R. D., De Vos, R. C. H., Mumm, R., & Hall, R. D. (2021). Metabolomics Reveals Heterogeneity in the Chemical Composition of Green and White Spears of Asparagus (A. officinalis). *Metabolites, 11*(10), 1–19. https://doi.org/10.3390/metabo11100708

Popa, S., Murith, N. M., Chisholm, H., Engmann, J., Popa Nita, S., Murith, M., … Engmann, J. (2013). Matching the Rheological Properties of Videofluoroscopic Contrast Agents and Thickened Liquid Prescriptions. *Dysphagia, 28*(2), 245–252. https://doi.org/10.1007/s00455-012-9441-x

Sagar, V. R., Suresh Kumar, P., & Suresh Kumar, S. V. R. (2010). Recent advances in drying and dehydration of fruits and vegetables: a review. Issue 1. In *Mysore J Food Sci Technol*. Springer-Verlag. https://doi.org/10.1007/s13197-010-0010-8.

Siccama, J. W., Pegiou, E., Eijkelboom, N. M., Zhang, L., Mumm, R., Hall, R. D., & Schutyser, M. A. I. (2021). The effect of partial replacement of maltodextrin with vegetable fibres in spray-dried white asparagus powder on its physical and aroma properties. *Food Chemistry, 356*(December 2020), 129567. https://doi.org/10.1016/j.foodchem.2021.129567

Siccama, J. W., Pegiou, E., Zhang, L., Mumm, R., Hall, R. D., Boom, R. M., & Schutyser, M. A. I. (2021). Maltodextrin improves physical properties and volatile compound retention of spray-dried asparagus concentrate. *LWT - Food Science and Technology, 142*. https://doi.org/10.1016/j.lwt.2021.111058

Ssepuuya, G., Katongole, J., & Tumuhimbise, G. A. (2018). Contribution of instant amaranth (Amaranthus hypochondriacus L.)-based vegetable soup to nourishment of boarding school adolescents. *Food Science and Nutrition, 6*(6), 1402–1409. https://doi.org/10.1002/fsn3.664

Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., … Viant, M. R. (2007). Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics, 3*(3), 211–221. https://doi.org/10.1007/s11306-007-0082-2

Thevenot, E. A., Roux, A., Xu, Y., Ezan, E., & Zunot, C. (2015). Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. *Journal of Proteome Research, 14*(8), 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354

Tressl, R., Holzer, M., & Apetz, M. (1977a). Formation of Flavor Components in Asparagus. 1. Biosynthesis of Sulfur-Containing Acids in Asparagus. *Journal of Agricultural and Food Chemistry, 25*(3), 455–459.

Tressl, R., Holzer, M., & Apetz, M. (1977b). Formation of Flavor Components in Asparagus. 2. Formation of Flavor Components in Cooked Asparagus. *Journal of Agricultural and Food Chemistry, 25*(3), 455–459. https://doi.org/10.1021/jf60211a025

Tyle, P. (1993). Effect of size, shape and hardness of particles in suspension on oral texture and palatability. *Acta Psychologica, 84*(1), 111–118. https://doi.org/10.1016/0001-6918(93)90077-5

Ulrich, D., Hoberg, E., Bittner, T., Engewald, W., & Meilchen, K. (2001). Contribution of volatile compounds to the flavor of cooked asparagus. *European Food Research and Technology, 213*(3), 200–204. https://doi.org/10.1007/s002170100349

Utpott, M., Rodrigues, E., Rios, A.de O., Mercali, G. D., & Flôres, S. H. (2022). Metabolomics: An analytical technique for food processing evaluation. *Food Chemistry, 366*(February 2021). https://doi.org/10.1016/j.foodchem.2021.130685

Wehrens, R., Hageman, J. A., van Eeuwijk, F., Kooke, R., Flood, P. J., Wijnker, E., … de Vos, R. C. H. (2016). Improved batch correction in untargeted MS-based metabolomics. *Metabolomics, 12*(5). https://doi.org/10.1007/s11306-016-1015-8

Whitson, M. E., Miracle, R. E., & Drake, M. A. (2010). Sensory characterization of chemical components responsible for cardboard flavor in whey protein. *Journal of Sensory Studies, 25*(4), 616–636. https://doi.org/10.1111/j.1745-459X.2010.00289.x