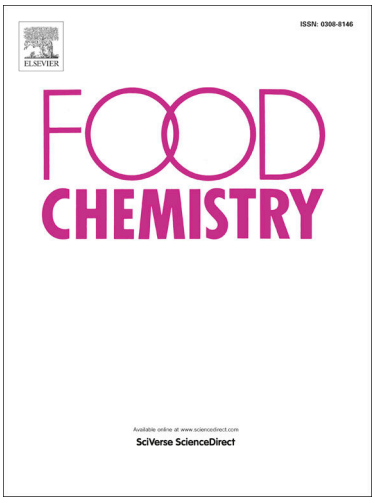
Journal Pre-proofs



Phytochemical characterisation and aromatic potential for brewing of wild hops (*Humulus lupulus* L.) from Northern France: Towards a lead for local hop varieties

Anne-Sophie Paguet, Ali Siah, Gabriel Lefèvre, Mathilde Vandenberghe, David Lutun, Norman Degardin, Jennifer Samaillie, David Mathiron, Charles Dermont, Franck Michels, Marie-Laure Fauconnier, Sylvie Chollet, Roland Molinié, Jean-Xavier Fontaine, Sevser Sahpaz, Céline Rivière

PII: S0308-8146(23)01920-9
DOI: https://doi.org/10.1016/j.foodchem.2023.137302
Reference: FOCH 137302

To appear in: *Food Chemistry*

Received Date: 7 June 2023
Revised Date: 24 August 2023
Accepted Date: 25 August 2023

Please cite this article as: Paguet, A-S., Siah, A., Lefèvre, G., Vandenberghe, M., Lutun, D., Degardin, N., Samaillie, J., Mathiron, D., Dermont, C., Michels, F., Fauconnier, M-L., Chollet, S., Molinié, R., Fontaine, J-X., Sahpaz, S., Rivière, C., Phytochemical characterisation and aromatic potential for brewing of wild hops (*Humulus lupulus* L.) from Northern France: Towards a lead for local hop varieties, *Food Chemistry* (2023), doi: https://doi.org/10.1016/j.foodchem.2023.137302

Phytochemical characterisation and aromatic potential for brewing of wild hops (*Humulus lupulus* L.) from Northern France: towards a lead for local hop varieties

Anne-Sophie Paguet[1], Ali Siah[1], Gabriel Lefèvre[1], Mathilde Vandenberghe[1], David Lutun[2], Norman Degardin[2], Jennifer Samaillie[1], David Mathiron[3], Charles Dermont[1], Franck Michels[1], Marie-Laure Fauconnier[1], Sylvie Chollet[1], Roland Molinié[1], Jean-Xavier Fontaine[1], Sevser Sahpaz[1], Céline Rivière[1]*

[1] Joint Research Unit 1158 BioEcoAgro, University of Lille, Junia-ISA, University of Liège, University of Picardy Jules Verne, University of Artois, ULCO, INRAE, Villeneuve d'Ascq, France

[2] High School Biotech Douai - Campus Wagnonville, 458 Rue de la Motte Julien, 59500 Douai, France

[3] University of Picardie Jules Verne (UPJV), Analytical Platform, Rue Dallery – Passage du sourire d'Avril, 80039 Amiens cedex, France

* Corresponding author: celine.riviere@univ-lille.fr

Abstract

In the current context of developing aromatic beers, our study aims at deciphering the chemical characterisation of cones from 39 wild hop genotypes collected in the North of France and replanted in an experimental hop farm, as well as 10 commercial and 3 heirloom varieties, using HS-SPME/GC-MS for the volatile compounds, UHPLC-UV for phenolic compound quantification, and UHPLC-IMS-HRMS for untargeted metabolomics. These analyses revealed a strong opposition between wild accessions and reference varieties, and an original chemical composition of some genotypes. 27 beers were produced with the same recipe, analysed by SBSE-GC-MS and evaluated by panellists. The unique difference relates to the hops to be assessed in order to determine their sensory profile. The different datasets were compared by OPLS-DA analysis in order to identify chemical markers which may influence the hop aromatic potential. Our results highlight the aromatic potential of some wild accessions, close to the commercial variety Cascade.

Keywords: *Humulus lupulus* L.; wild hops; chemodiversity; sensory analysis; mass spectrometry; multivariate analysis

1. Introduction

Beer is a fermented drink, which has traditionally been flavoured with hop (*Humulus lupulus* L., Cannabaceae) since the Middle Ages. Female inflorescences, commonly referred as hops or hop cones, are added to the wort for their bitterness and their aromatic originality as well as for their antiseptic properties, linked to their original chemical composition (De Keukeleire et al., 1992). Original phenolic compounds, including prenylated chalcones as xanthohumol and desmethylxanthohumol and acylphloroglucinol derivatives (α-acids or humulone derivatives and β-acids or lupulone derivatives), are produced in lupulin glands at the base of the bracts. Hop essential oil is also rich in non-oxygenated monoterpenes and sesquiterpenes, among them β-myrcene, α-humulene and β-caryophyllene (Bocquet et al., 2018). When bittering hops are added to the wort during the boiling, α-acids are isomerised into iso-α-acids, in *cis* or *trans* position (Schönberger & Kostelecky, 2011) and provide up to 80% of a beer's bitterness (De Keukeleire et al., 1992). By contrast, aromatic hops are rather added to the wort at the end or after its boiling, to minimise the evaporation of their volatile compounds (Sharpe & Laws, 1981). Monoterpenes and sesquiterpenes are considered to be responsible for the "hoppy" aroma of beer (Van Opstaele et al., 2010). α-humulene and β-caryophyllene can impart spicy and woody notes to beer in particular due to the formation of oxygenated derivatives during wort boiling. β-myrcene, an important contributor to fresh hop aroma, is largely lost during beer processing except in dry hopping, but oxygenated derivatives formed can contribute to the fragrance. Among other terpenoids, alcohol monoterpenes (linalool, geraniol, β-citronellol, α-terpineol) and aldehyde monoterpenes (citral) provide floral and citrus notes to beer, respectively (Rettberg et al., 2018). By contrast, other fruity aroma can be attributed to the presence of some esters, such as red berries-like for ethyl-3-methylbutanoate, whereas aldehydes, such as hexanal, confer grassy fragrances to the beer (Machado et al., 2021). Perception of hop volatile compounds after brewing remains complex. Interactions between hop volatile compounds and other beer components (ethanol, carbohydrates, yeast, hop bitter acids…), combined to oxidation phenomena, require a thorough investigation to understand their resulting effects on the final sensory profile of beer (Dietz et al., 2020). Hops also play a role in the characteristics and stabilisation of the foam (De Keukeleire et al., 1992). The hydroxyl group of the isohumulone acts as a surfactant, which helps to strengthen the electrostatic bonds between the bubbles and thus stabilises the foam (Asano & Hashimoto, 1980).

Northern France is an historical region for beer craft and hop production. In the beginning of the XXth century, 1220 ha of hops and nearly 2,000 breweries were inventoried in the region (Ducloux et al., 1910). If beer production remains important (around 6 Bn hL in 2020), hop production for its part clearly decreased during the XXth century, to reach only 35 ha today (Bart-Haas Group, 2021). This decline can be explained by the economic context of the last century, when French brewers started to import hops from the USA or Germany. However, in the context of the "beer craft movement", current brewers have been looking for local and aromatic hops from sustainable or organic agriculture for a decade (Paguet et al., 2022). In this context, the objective of our study is to investigate the diversity of wild hops from Northern France, that can be used further to support a future varietal development (Paguet et al., 2022). In a previous study, we investigated the genetic and the chemical diversity of 50 wild hop accessions collected *in-situ* from eleven natural sites of the North of France, in the Hauts-de-France region (Paguet et al., 2023). These wild accessions were compared to ten commercial varieties and three old varieties coming from the same region for their genetic and chemical characteristics. This study underlined a high genetic diversity and chemical variability among wild accessions (Paguet et al., 2023). During the collection of samples, rhizomes were also collected and transplanted into our experimental field to get a germplasm resource. The goal of the current study was to assess these accessions in *ex-situ* conditions and to analyse their chemical diversity under more standardised conditions. The more productive accessions were used for the production of beers using the same beer style (lager style). The only difference in the recipe was the aromatic hops, with wild or commercial origin. The beers were subjected to a sensory study in order to highlight the aromatic traits of these hops after brewing. Recently, Machado et al., (2021) have studied the aromatic qualities of wild hops

on dry-hopped beers. Hong et al. (2022) evaluated, using partial least-squares discriminant analysis, the link between chemical characteristics of cones and their influence in hop-tea or after brewing.

While our previous article Paguet et al. (2023) concerned wild hop accessions collected under *in*-situ conditions, the present study deals with these same accessions replanted in our experimental hop field, and therefore constitute a new dataset. The chemical composition of wild hop accessions, grown under *ex-situ* conditions, and their aromatic potential were determined using combined analytical methods, including HS-SPME GC-MS, UHPLC-UV and UHPLC-IMS-HRMS. The physico-chemical characterisation of the beers was carried out using analytical methods such as SBSE GC-MS, while a panel of experts was used to characterise the beers according to a certain range of descriptors. Moreover, sensory evaluation and multiple statistical methods (heatmap, principal component analysis (PCA), orthogonal partial least squares discriminant analysis OPLS-DA) were carried out to explore the relationship between wild hops' chemical composition and beer aroma.

2. Material and methods

2.1. Samples

2.1.1. Hop collection

50 wild hops coming from 11 different locations, with ecological or ethnobotanical interests, were harvested in September 2019, in accordance with the rules of the Nagoya Protocol and the French biodiversity law of 2017 (decision of June 9, 2020 issued by the Ministry of Ecological and Inclusive Transition; NOR: TREL2002508 S/284) (Paguet et al., 2023). The rhizomes of these accessions had also been collected and transplanted in November 2019 into our experimental hop field (Ferme brasserie Wagnonville, Douai, France). The present study focuses on the characterisation of the 32 most productive accessions in cones to enable their analysis among the 50 that had been replanted. Cones were collected at their maturity (80% humidity) in September 2021, oven-dried at a temperature below 40°C until they reached a moisture content of 10% and then stored under vacuum at -20°C before being used for analysis. These wild accessions cultivated in field conditions were compared to 10 commercial cultivars grown in the North of France region. They were selected among the ten most relevant varieties, including so-called top hops used by craft brewers, such as Brewers Gold, Cascade, Challenger, Fuggles, Goldings, Magnum, Northern Brewer, Nugget, Strisselspalt and Target. Cones of commercial hops, collected in September 2021, were provided by the Northern France Hop Cooperative, Coophounord (Bailleul, France). They were also compared to three heirloom varieties collected the same year. Three genbank clones representing former Flander cultivars were included: Groene Bel, Coigneau, and Record, coming from Belgium (Hoppecruyt, Poperinge, Belgium). Dried hop cones, kept vacuum-packed in cold storage, were grounded in liquid nitrogen using a blender IKA A11 (Staufen, Germany) before being used for extraction.

2.1.2. Beer samples: Brewing processes

To investigate the aromatic potential of these wild hop accessions after brewing, the 17 more productive hops of our collection were brewed, according to a recipe of blond Pilsner, for chemical characterisation and sensory evaluation. These beers were compared to beers elaborated with 2 old varieties (Groene Bel and Record) and seven commercial varieties (Nugget, Strisselspalt, Cascade, Magnum, Brewers Gold, Target and Northern Brewers. The mash was brewed in 60 L in mash-in with 13 kg of Pilsen malt (La Malterie du Château, Beloeil, Belgium) at 67°C for 1 hour and 15 minutes and filtered. Afterwards, the wort was boiled and 30 grams of bittering hops cv. Magnum in pellets (Coophounord, Bailleul, France) were added 10 minutes after the beginning of boiling. After boiling, the wort was divided into 6 brew tests of 10 L each for aroma hopping. 50 grams of dried hops to evaluate, the only ingredient differentiating the brews, were added. A beer control without aromatic

hop was also performed. Aromatic hops were added after the boiling step in infusion during the intonation for 20 min (steeping/whirpool hops mode). After cooling, fermentation took place at atmospheric pressure at 10°C (low fermentation) for one week, with *Saccharomyces cerevisiae* LalBrew Nottingham yeasts (Lallemand Brewing, Felixstowe, United Kingdom). Lagering was carried out at 4°C for 10 days. The beers were then racked in bottles, without filtration or pasteurisation.

2.2. Chemical analysis

2.2.1. Volatile compound analysis

2.2.1.1. Volatile compound analysis in hops by HS-SPME GC-MS

Extraction of volatile compounds by HS-SPME was reached using a triple-phase fiber, 50/30µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA), that was preconditioned according to the instructions of the manufacturer. Extraction was done on 2 grams of hop cone powder placed in a 20 mL vial incubated at 45°C for 5 min. We used the same chromatographic conditions as those detailed in Paguet et al., 2023, on an Agilent 7890 A gas chromatograph coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with an MPS auto-sampler and an HP-5 MS capillary column (30 m × 250 µm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). Injections were performed in splitless mode at 280 °C and helium was employed as the carrier gas at a flow rate of 1.2 $mL.min^{-1}$. The separation conditions were as follows: initial column temperature of 40°C for 2 min; then the temperature was successively increased by 4°C/min up to 200°C then by 20 °C/min up to 300°C, where it was maintained for 5 min. The mass spectrometer was set to have a temperature of the ion source at 230°C and was programmed with SCAN acquisition mode.

2.2.1.2. Beer analysis by SBSE GC-MS.

Ten milliliters of beer samples were taken and placed in a vial with 2 grams of NaCl (VWR, France) adding a stir bar (10mm x 0.5mm) with PDMS coating (SBSE Gerstel-Twister, USA) and stirring for 120 min at 1000 rpm. Some samples were analysed twice on two different bottles, to ensure the inter-bottle repeatability. After extraction, the stir bar was rinsed with some distilled water, dipped on a filter paper and introduced in a glass thermal desorption tube (4 mm i.d. x 178 mm L). The stir bar was then placed in the thermal desorption unit of an Agilent 7890 GC coupled to an Agilent 5975 C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a MPS auto-sampler and a HP-5 MS capillary column (30 m x 0.25 mm x 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven was programmed as follows: initial temperature of column of 40°C with a hold of 2 minutes; then increased by 6°C/min to 300°C, where it was maintained for 5 min. Stir bars were thermally desorbed by programming the system from 40°C to 260°C with a rate of 6°C/min and held at this temperature for 5 min. The desorbed analytes were cryofocused at -10°C. Injection was performed in the splitless mode. These analyses are intended to verify intra-batch homogeneity and inter-batch variations.

2.2.1.3. Volatile compound identification

MassHunter Version B.06.00 (Agilent Technologies, Santa Clara, CA, USA) was used for data acquisition and processing. General volatile compound profiles were established through a chromatographic deconvolution process (Agilent MassHunter Unknowns Analysis) and chromatographic areas were obtained for each volatile compound. Identification of the individual components was based on: (a) comparison of the mass spectrum (MS) outcomes to those of commercial databases: National Institute of Standards and Technology (NIST17) and Wiley7 (match factor threshold > 700); (b) comparison of the retention index (RI) of each peak with literature RI data (± 20) from the NIST WebBook. Experimental retention index (RI) of the compounds were calculated following the injection of a mixture of n-alkanes C8-C20 (Sigma Aldrich, Darmstadt, Germany). They

were first reported as a percentage of the total chromatographic area on Excel (Microsoft Excel 2016) to allow a general analysis.

2.2.2. Analysis of non-volatile compounds

2.2.2.1. Sample preparation

For each hop studied in our study, hydro-ethanolic extracts were performed on 50 mg of dried cone powder with 1 mL of ethanol-water mixture (9:1, v/v). A one-hour maceration session was carried out in an ultrasonic bath. Afterwards, tubes were centrifuged at 4000 rpm at 20°C for 5 min. Supernatant was then transferred in a tared tube. The exhausted matrix was then re-extracted two times more following the same protocol and each time the supernatant was pooled with the first extract. At the end of the three maceration sessions, extracts were then evaporated in a centrifugal concentrator Genevac™. Extracts were diluted at 1 mg/mL in methanol (Carlo Erba), filtered through 0.22 µm PTFE membrane and placed in glass vials for further LC-MS analysis. Quality control (QC) samples were prepared by pooling 10 µL from each sample of the same round preparation and thoroughly mixed.

2.2.2.2. Setting up and validation of the quantification method of main phenolic compounds

Quantification of xanthohumol, *co-,* n- and *ad*-humulone, *co-,* n- *and ad*-lupulone in each hydro-ethanolic extract was performed on an Acquity UPLC® H-Class Waters® system (Waters, Guyancourt, France) coupled with a Diode Array Detector (DAD) and a QDa ESI-Quadrupole Mass Spectrometer. Separation was achieved using a Waters® Acquity BEH C18 column (pore size 300 Å, particle size 1.7 µm, 2.1 x 50 mm, Waters, Milford MA) connected to a 0.2 µm in-line filter. Solvent A (water with 0.1% formic acid, v/v) and solvent B (acetonitrile with 0.1% formic acid, v/v) were used as mobile phases. Compounds were eluted using the following chromatographic conditions: the flow rate was 0.3 mL/min; the column temperature was set at 30°C; the injection volume was 2 µL. The gradient elution was performed using eluent A and eluent B: initial condition at 50% B, 0-1 min isocratic step, 1-3 min linear gradient to 75% B, 3-5 min isocratic step at 75% B, 5-7 min linear gradient to 100% B, 7-9 min isocratic step at 100% B, 9-9.5 min linear gradient to 50% B, 9.5–13 min isocratic step at 50% B (total analysis time: 13 min). The ionisation was performed in negative mode. Cone voltage was set at 10 V. Probe temperature was 600 °C. Capillary voltage was 0.8 kV. The MS-Scan mode was used from 100 to 1000 Da. Xanthohumol and acylphloroglucinol derivatives were quantified according to the International Conference for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guideline Q2-R1 (ICH, 2005). Quantification was performed in UV at 370 nm for xanthohumol and 330 nm for acylphloroglucinols. The quantitation method was set up using standards purified in the laboratory according to the protocol detailed by Bocquet et al. (2019) and following the methodology detailed in Paguet et al. (2023). *Ad*-humulone and *ad*-lupulone were also quantified by the establishment of calibration range using standards purified in the same way as the one detailed in Bocquet et al. (2019) for *n*- and *ad*- humulone and lupulone. The quantitation of each compound was performed in five technical replicates. QC samples were analysed regularly along with unknown samples. To reduce the effects of systematic errors, all samples were assigned to a random LC–MS run order and interspersed after every 20 injections with QC sample injections. Xanthohumol and α- and β-acids were identified based on the retention time of purified standards and their mass spectra and quantified using the quantification methods of previously set up on the Empower 3 software. Data were exported from Empower 3 to Excel (Microsoft Excel 2016).

2.2.2.3. UHPLC-IMS-HRMS analysis

UHPLC-IMS-HRMS analysis were performed using a Waters ACQUITY UPLC I-Class system interfaced with a Vion IMS Q-TOF (Ion Mobility Quadrupole Time-of-flight) hybrid mass spectrometer,

equipped with an electrospray ionisation (ESI) source (Waters, Manchester, UK). The autosampler was programmed to inject 2 µL of each sample. Chromatographic separation of the analytes was carried out using the same column, a Waters® Acquity BEH C18 column (2.1 × 50 mm, 1.7 µm, Waters, Milford MA) connected to a 0.2 µm in-line filter maintained at 40°C. The UHPLC method was carried out following the same protocol and procedure described previously for the quantification: the flow rate was 0.3 mL/min; the column temperature was set at 30°C; solvent A (water with 0.1% formic acid, v/v) and solvent B (acetonitrile with 0.1% formic acid, v/v); the gradient was the following: initial conditions at 50% B, 0-1 min isocratic mode, 1-3 min linear gradient to 75% B, 3-5 min isocratic mode at 75% B, 5-7 min linear gradient to 100% B, 7-9 min isocratic mode at 100% B, before re-equilibration time of 4 min. For HRMS, the ESI parameters were set as follows: capillary voltage, 2.4 kV in negative mode; source temperature, 120°C; desolvation temperature of 450°C. Time-of-flight (TOF) MS was operated in sensitive mode. The data were acquired in high-definition MS^E ($HDMS^E$) over a mass range of *m/z* 50-1200 at a mass resolving power of 50,000 FWHM and a scan time of 0.2 s. Spectra were acquired and processed with the UNIFI software (version 1.9.4, Waters), allowing to generate the data matrix with default parameters comprising the retention time, mass-to-charge ratio (*m/z*) values, and peak intensity. The untargeted metabolomic data matrix was cleaned by removing the variables with a significant variance (>35%) in the quality control (QC) and the variables present in the blank. A minimum intensity threshold was chosen at 500 to keep the variable. Metabolites were principally identified by matching the accurate masses, retention times and fragmentation patterns with those of the reference standards and literature references. Supplementary analyses in DDA mode were done, with a collision energy of 20 eV on QC to perform dereplication analysis on UNIFI and MassLynx (version 4.1, Waters) softwares.

2.3. Beer characterisation

2.3.1. Physico-chemical characterisation of beers.

Beers were characterised according to different physico-chemical parameters following the American Society of Brewing Chemists recommandations (1992). Samples were preliminarily degassed by agitation in the open air.

(i) Beer colour (EBC). Beer colour was determined by an official method from ASBC, in which the absorbance of beer samples was measured at 430 nm using a spectrophotometer UV-1280 (Shimadzu, France). EBC was achieved following equations: $EBC = 50*D*Abs_{430nm}$, where D is the dilution factor, and Abs_{430nm} the absorbance at 430 nm.

(ii) Bitterness dosage (IBU). Bitterness was determined following Kawa-Rygielska et al. (2019) method: 10 mL of beer sample was added to 1 mL of HCl and 20 mL of isooctane. Tubes were stirred for 15 min and centrifuged at 1880 G for 3 min. The supernatant was measured at 275 nm against a blank control (pure isooctane) with a spectrophotometer UV-1280 (Shimadzu, France). IBU values were achieved as follows: $IBU = Abs_{275nm}*50$ where IBU is the International Bitterness Unit, and Abs_{275nm} is the absorbance at 275 nm.

(iii) Determination of total acidity. Acidity of beer is considered as mainly due to the presence of malic acid, measured by acid-base titration with soda NaCl 0.1 M, using phenolphthalein as indicator of equivalence.

(iv) Wort density and alcohol content. Wort density (expressed in °Plato) and alcohol content (% v/v) were measured with a densimeter Alex 500 (Anton Paar, France) on 50 mL of beer sample vacuum filtered through Kieselguhr. Repeatability of bottles was tested using a Student test.

2.3.2. Sensory characterisation of beers

2.3.2.1. Panel training

Twenty-one trained subjects (13 men and 8 women) were enrolled in a training program designed to produce beer experts. They were trained 1hour a week to describe beers on seventeen descriptors (intensity of the smell, hoppy smell, malty smell, fruity smell, intensity of taste, acid, bitter, floral, hops, citrus, malt, sweet, spicy, red fruits, yellow fruits, astringent and bitter persistence) and to evaluate the intensity of these descriptors on a non-structured linear scale. These descriptors have been chosen in the literature and were recalled by the panelists during vocabulary generation sessions. At the time of the experiment, the panelists had already received 12 hours of training. At this point, they should have developed a consensual vocabulary, along with the ability to detect the descriptors on which they have been trained.

2.3.2.2. Procedure

The assessors evaluated the 26 beer samples (17 with wild hops, 7 with commercial varieties, 2 with old varieties) in duplicate during 12 sessions under standard sensory conditions (ISO, 2016). Samples were presented in transparent glasses and 20 mL were served between 8 and 10°C, in a sequential monadic way. Their presentation order was different for each assessor and based on a Williams' Latin-square arrangement. Subjects had to evaluate beers on the 17 descriptors according to a non-structured linear scale from 0 to 10 using FIZZ software (Biosystemes, Dijon, France). The significant descriptors were identified with three factors ANOVA (product, repetition and panelist), measuring the *p*-value on the factor product.

2.4. Data analysis

For each simple dataset collected, data were compiled into an Excel sheet. Statistical analysis as Principal Component Analysis (PCA) for quantitation of prenylated phenolic compounds, untargeted metabolomics and sensory analysis, Agglomerative Hierarchical Clustering (AHC) for composition of volatile compounds and sensory analysis, as well as heatmaps for composition of volatile compounds and quantitation of phenolic compounds were achieved using XLStat (Addinsoft, 2022). On the quantitation triplicate, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with $p < 0.05$ was conducted using XLStat (Addinsoft, 2022). Student tests for the verification of the inter-bottles repeatability and the choice of the selection of the most significant descriptors were achieved using the statistical tests on XLStat (Addinsoft, 2022).

To link analytical measurement and sensory analysis, OPLS-DA model was performed using MATLAB R2014a (MathWorks, Natick, MA) software. OPLS-DA model provide insights into separations between experimental groups, here based on sensorial analysis discrimination results, based on analytical measurements (here the phytochemical characterisation of cones). Hence, the OPLS model comprises two blocks of model variations: 1) the Y-predictive block, which represents the variation between the classes, and 2) the Y-orthogonal block also referred to the uncorrelated variation, which constitutes the within class variation (Bylesjö et al., 2007). As we preferred to focus on the link between cone phytochemistry and panel-assessed beer aromas, the SBSE-GC-MS analysis dataset was not included in our orthogonal analysis.

3. Results

3.1. Chemical characterisation of cones

3.1.1. Volatile compound analysis by HS-SPME GC-MS

The putative identification of volatile compounds by HS-SPME GC-MS revealed the presence of 65 different compounds among hops studied in the study. Thirty-two compounds were detected in

fixing a threshold at 1%. This volatile compound analysis was represented by a heatmap and a dendrogram **(Figure 1)**. The majority of these compounds were monoterpenes or sesquiterpenes **(Table S1)**. In particular, we identified β-caryophyllene, β-myrcene and α-humulene, known as the main volatile compounds of hops, in all accessions characterised **(Figure 1A)**. The dendrogram associated with the AHC (**Figure 1B)** distinguished 4 different classes of accessions according to their content of volatile compounds. The blue cluster was mainly composed of commercial and old cultivars, as well as wild accessions I3 and G2. These accessions were characterised by a high content of the main hop volatile compounds, as α-humulene, β-myrcene and β-caryophyllene. In **Figure 1B,** we then noted that the commercial accessions Cascade, Fuggle and Strisselspalt, known for their aromatic characteristics, were distributed in different clusters from other commercial accessions known for their bitter potential. The purple cluster, including Strisseltspalt, showed a high level of other volatile compounds, such as limonene, germacrene and β-elemene. The green and purple classes, represented by different accessions, both wild, old and commercial, as well as the red class, only composed of a few wild accessions, showed a lower content of the majority non-oxygenated terpenes (α-humulene, β-myrcene and β-caryophyllene). By contrast, they showed higher contents of minor volatile compounds that were not taken into account in this qualitative classification as γ-muurolene (for B4 and H4 in the purple cluster) or α-cedrol (for I8 in the green one), known for their woody aromas **(Table S1)**. We also identified the presence of some particular sesquiterpenes in both wild and commercial accessions, such as α-bergamotene (in Cascade, Fuggle, H2, H3 and I8) or β-farnesene, α- and β-selinene, or even β- and γ- elemene mostly present in wild accessions **(Figure 1A).**

3.1.2. Quantification of main phenolic compounds by UHPLC-UV

UHPLC-UV analysis revealed the presence of seven main peaks at the retention times 2.76, 4.35, 4.73, 4.83, 6.26, 6.87 and 7.00 minutes attributed to xanthohumol (XN), α-acids *(co-*, *n-*, *ad-*humulone) and β-acids (*co-*, *n-*, *ad-*lupulone) respectively. This attribution was based both on their retention times compared with those of the standards purified in the laboratory and on their mass spectra. Xanthohumol, *co*-humulone, *n*-humulone, *ad*-humulone, *co*-lupulone, *n*-lupulone and *ad*-lupulone were then quantified in 52 crude extracts of cone samples using the quantitation method setting up by UHPLC-UV. Acceptable linearity was observed for each compound over the concentration range used for calibration **(Table S2)**. Evaluation of the recovery data of the quantification method showed acceptable intra and inter-day precisions for xanthohumol (RSD % = 18.64, 13.69), *co*-humulone (RSD % = 13.13, 6.40), *n*-humulone (RSD % = 4.01, 2.55), *ad*-humulone (RSD % = 15.55, 16.58), *co*-lupulone (RSD % = 10.19, 11.17), *n*-lupulone (RSD % = 16.12, 9.7) and *ad*-lupulone (RSD % = 15.15, 16.68). Results were expressed in µg/mL and converted in percentages of dry matter. Statistical analysis with a Tukey's test revealed significant differences among hop samples in terms of composition in phenolic compounds **(Table S3)**. Overall, commercial varieties showed a higher content of *n*-humulone, *ad*-humulone and xanthohumol, which may assert their bitter potential, unlike in wild accessions which produced more β-acids. Nevertheless, the cultivars Strisselspalt and Fuggle were distinguished by low contents of phenolic compounds than other commercial varieties. On the other side, some wild hops showed higher levels than commercial varieties. In the detail, higher contents in xanthohumol, total α-acids and total β-acids were observed for the accessions Target (0.045 %), Magnum (2.748 %) and Groene Bel (3.138 %) respectively. Lower contents were observed for C3 (0.005 %), D1 (0.100%) and I5 (0.353 %) respectively **(Figure 2A and Table S3)**. **Figure 2A** thus revealed low contents of humulone for wild hops compared to commercial varieties. Some exceptions were noted for the wild hops I3, I1, G2, B3, D3 and J3 that showed contents of humulone similar to commercial or old cultivars (from 1.274 % to 0.621 %). Wild hops also displayed low contents of *ad*-humulone and xanthohumol. By contrast, most wild accessions had levels of *co*-humulone and β-acids which were not negligible. These phenolic compounds will have a different impact on bitterness. The output process of the quantification represented in the form of PCA **(Figures 2B and 2C)** revealed a good explanation of the distribution of variables, equal to 92.32 %, with a contribution of PC1 and PC2 equal to 72.48 % and 19.84 % respectively. Moreover, the biplot clearly revealed a good correlation between

α-acid and xanthohumol contents on one side and β-acid contents on the other side **(Figure 2C)**. According to this figure, most of the commercial and heirloom varieties had higher contents in *n*-humulone*, ad*-humulone and xanthohumol. On the contrary, wild hops (Locations A to K) and the old variety Groene Bel showed higher *б*-acid contents (*co*-, *n*- and *ad*-lupulone). The strong separation between commercial cultivars and wild hops was also illustrated by the dendrogram **(Figure S1)**. The colouration of the accessions, on the biplot and on the dendrogram, based on their clustering previously achieved with their composition in volatile compounds, allowed us to evaluate the composition both in main phenolic compounds and in volatile compounds. Except for the old variety Groene Bel, this new biplot showed that the accessions were globally grouped according to their colour, and therefore according to their composition in volatile compounds **(Figure 2B)**. This study also underlined that the accession I3 had a chemical composition very close to those of commercial varieties because it belonged to the same cluster both for GC-MS analysis and quantification by UHPLC-UV **(Figure 1B and Figure S1).**

3.2.2.3. Dereplication and untargeted metabolomics by UHPLC-IMS-HRMS

From 164 601 variables acquired, we finally kept 262 variables (160 variables from 1 to 7 min) after cleaning the matrix. From these 160 variables, 15 compounds were identified based on previous works including Farag et al. (2012) and Nicácio et al. (2022) **(Table S4)**. These partially annotated metabolites were classified as putative MS/MS annotations and one compound was labeled as an unknown compound **(Figure 3A)**. Among them, the 7 compounds previously quantified (xanthohumol, *co*-, *n*- and *ad*-humulone and *co*-, *n*- and *ad*-lupulone) were also identified using PDA spectrum and retention times. As presented in the **Table S5** and on the **Figure 3C**, different classes of metabolites were represented, in particular chalcones and flavanones [desmethylxanthohumol (1), xanthohumol (2), isoxanthohumol (3)], as well as acylphloroglucinol derivatives including *α*-acids and oxidised derivatives [cohumulone (5), 4-deoxycohumulone (6), humulone (7), humulinone (8), adhumulone (9), deoxyhumulone (10), préhumulone (11)] and β-acids [postlupulone (12), colupulone (13), lupulone (14), adlupulone (15), prelupulone (16)]. **Figures 3B and C** showed the PCA score plots for data acquired in HRMS in negative ion mode corresponding to the UHPLC retention times ranging from 1 to 7 min. Considering the two principal components, we explained 44.78% of the variance distributed as follows: 28.31% for the PC1 and 16.47% for the PC2. On **Figure 3B**, individuals were again coloured according to their composition in volatile compounds after the AHC shown on the **Figure 1B**. Thus, we noted that we globally had the same repartition of blue varieties after PCA treatment on the UHPLC-HRMS data; while accessions from other groups were more mixed. The identification of some compounds on **Figure 3C** can provide some explanations to this distribution. Individuals belonging to the first cluster coloured in blue, which gathered most of the commercial and old varieties (in square), globally showed higher contents in *α*- and *б*-acids. **Figure 3C** also highlighted that chemical families, including chalcones, *α*- and *б*-acids, were close on the loading plot. These results therefore allowed the identification of some accessions with high contents of *α*-acids, not only in *co*-, *n*- and *ad*-humulone previously quantified. We have previously identified two accessions, I3 and G2, with a composition in volatile compounds and *α*-acids close to those of commercial varieties. **Figures 3D and 3E**, only focused on wild accessions, confirmed the high content of *α*-acid derivatives in these two accessions. These figures also allowed us to better compare wild accessions with each other. It hence appeared that wild accessions, with a composition in volatile compounds close to those of commercial varieties (in red and in blue), had a lower content of acylphloroglucinol derivatives, while some wild accessions, as the accessions A5, B2, B3, I1 or H1, had a higher content of these compounds. However, it would be relevant to continue the identification of the chemical markers located at the top of PC2 and which differentiate several wild individuals. The distribution of individuals on the loading plot was strongly different from the biplot of the quantification **(Figure 2B)**, and the explanation of the variance was relatively weak (less than 50%), thereby reflecting the high chemical diversity of the accessions studied.

3.2. Chemical characterisation after brewing: beer analysis and sensory analysis

Once the hop accessions have been chemically characterised, we got interested in their aromatic and gustative potential after brewing by sensory analysis. The beers elaborated according to a same base beer style (lager) and with the different hops to evaluate were first characterised by physico-chemical analysis before being tasted by a panel. The main objective of these analyses was to characterise the matrix and to ensure the homogeneity of the batches tasted by the panelists.

3.2.1. Physico-chemical analysis

Physico-chemical characterisations were based on a measurement of IBU, EBC, alcohol content, residual sugars and total acidity. These analyses revealed IBUs ranging from 13.1 (Cascade) to 38.2 (Magnum); alcohol content from 4.69 (F1) to 6.6 (Groene Bel) % v/v; EBC from 8.2 (H2) to 20.8 (H1); total acidity from 4.1 (G1) to 8.1 (Northern Brewers); residual sugars from 1.190 (Target) to 2.3 °P (B3) **(Table S5)**. Higher IBUs were measured for beers brewed with commercial bittering hops (Brewers Gold, Magnum), while beers brewed with wild hops had IBUs comparable to those of beers brewed with commercial aromatic hops (Striesselspalt and Target). EBCs measured were those of a Lager beer, according to the Pilsen malt used. Alcohol content, total acid concentration and density were not statistically influenced by the hop used for the brewing. Furthermore, student test (p = 0.95) revealed no significant differences among bottles of a same batch for alcohol, colour and residual sugars (with *p-value* respectively equal to 0.282, 0.618 and 0.223), while for total acidity, *p-value* was less than 0.0001, which indicated a high level of variability among bottles from a same batch **(Table S6)**.

3.2.2. Analysis of volatile compounds by SBSE GC-MS

In total, 180 volatile compounds were identified by SBSE GC-MS in beer samples. These compounds were mainly represented by the ester chemical class **(Figure S2)**. We also detected some sesquiterpenes, as α-humulene, β-caryophyllene, α-, β-, γ- and δ-selinene and alloaromadendrene **(Table S7)** previously identified in hop cones. Some monoterpenes, such as citronellol, were detected in beers but were not found in cones. The analysis of the beer brewed without aromatic hop revealed the quasi absence of terpenoids in this sample, and thus confirmed that terpenes and their derivatives present in other beer samples could come from hop tested. Lastly, analysis on several different bottles from the same batch revealed a similar composition in volatile compounds and thus confirmed the homogeneity of the flavours of the beers tasted by the panelists, even if they come from different bottles.

3.2.3. Sensory results

On the 17 descriptors on which the panel was trained **(Table S8)**, we finally retained nine descriptors which were significant (*p-value* <0.05%) **(Table S9)**. These descriptors were: hoppy smell, fruity smell, intensity of the taste, malt taste, hoppy taste, citrus taste, yellow fruits, bitter taste and bitter persistence. To process data by PCA, we kept the first two principal components explaining 75.06 % of the variance, and we considered the beer brewed with no aromatic hop as a supplementary observation **(Figure 4A)**. PC1 (56.27% of the variance explained) translated the intensity of the taste, bitter taste, bitter persistence, fruity smell, yellow fruits, and hoppy taste and smell; while PC2 (18.79%) translated the citrus and malt tastes **(Figure 4B)**. **Figure 4A** and **Figure 4B** highlighted the bitter taste of beers brewed with so-called bitter commercial varieties (such as Northern Brewers, Magnum, Brewers Gold or Target) which had previously revealed high α-acid levels. The HAC (**Figure 4C**) clearly opposed the cluster made of beers brewed with commercial varieties on one side (in blue) and another cluster composed of beers brewed with wild accessions on the other side (in red). The clear separation already observed for the chemical analysis of the 52 hop samples was thus confirmed after brewing (**Figure S3**). Nevertheless, the accessions I3 and G2 whose chemical composition was fairly close to the marketed varieties no longer belonged this time to the same cluster concerning the taste felt by the panelists after brewing. By contrast, in this sensory analysis, 5 wild accessions (B3, H1,

H2, I9, K5) of our collection belonged to the same cluster as commercial and old varieties **(Figure 4C)**. The chemical composition of these accessions was far from the reference varieties in regards to non-targeted metabolomic analysis. However, accessions H1, H2, K5 and I9 revealed the presence of some particular volatile compounds, while the accession B3 had high levels of some phenolic compounds. The proximity of the wild accessions H2 with the aromatic cultivar Cascade, and I9 with K5, observed on **Figure 4A,** had already been observed on the dendrogram **1B** for the analysis of volatile compounds. α-bergamotene was identified in accession H2, γ-elemene in accession K5 as well as β-elemene, γ-elemene, α-selinene, β-selinene and alloaromadendrene in accession I9. This composition was close to those of the accession Strisselspalt for example, also containing alloaromadendrene, α-selinene, β-selinene and γ-elemene and that could explain the fruity, hoppy and citrus perception of these accessions according to panellists. Concerning the chemical composition of beers analysed by SBSE GC-MS, some accessions (e.g. Brewers Gold, Magnum, B3, G1, I3 and I5) had a high level of monoterpenes and sesquiterpenes. Nevertheless, among these beers, those brewed with wild accessions did not show similar aroma profiles according to the panel. For the beer brewed with no aromatic hop (in yellow on **Figure 4A)**, analysed in the PCA as a supplementary observation, and therefore not included in the AHC, panelists did not discriminate it from the other samples tasted, and seemed to be fairly close to the accession G2 and the old variety Record, which may not have significant aromatic impact on the beer.

3.3. Discriminant analysis: OPLS-DA

To objectively assess the influence of the chemical composition of hop cones used for brewing in the final beer, a supervised OPLS-DA approach based on the chemical profiling and gustative evaluation was performed. Two classes were selected in this study, according to the results of the AHC of the sensory analysis: group 1 (n = 13 in blue) and group 2 (n = 13 in red) **(Figure 4C)**. In total, 225 variables identified in the two datasets previously collected to characterise the chemical diversity of hops (volatile compound analysis by HS-SPME GC-MS: 65 variables, untargeted metabolomic analysis by UHPLC-IMS-HRMS: 160 variables) were used for the OPLS-DA model to explain the aromatic diversity of the 26 accessions used in brewing tests. The combination of these data was expected to provide a global profiling of the gustative qualities of hops in an integrative brewing perspective. The consensus OPLS-DA strategy was applied for the differential analysis of the two aromatic groups and the simultaneous analysis of the two blocks of data.

The block contributions of the predictive latent variable indicated an equivalent importance of the volatile cone analysis by HS-SPME GC-MS (p: 51.91%, o: 52.05%) or of the untargeted metabolomics by UHPLC-IMS-HRMS (p: 48.08 %, o: 47.94 %) **(Figure S4)**. A model with one predictive and one orthogonal latent variable was evaluated as the best model based on the DQ2 value computed during leave one-out cross validation, i.e., DQ2 = 0.393. Hence, in view of this relative low value, the proposed model is an explanatory model and not a predictive model. Score plots and the contributions of the model were presented in **Figure 5**. This model confirmed the clear opposition between the two beer groups defined by the panelists, separated by the predictive component **(Figure 5A)** and used for the model construction. As discussed before, **Figure 5B** highlighted the influence of some volatile compounds on the aromatic profile of beers. Beers brewed with commercial varieties were influenced by their content of main hop volatile compounds (as α-humulene, β-myrcene, β-caryophyllene, linalool or β-pinene), while beers brewed with wild accessions were more distinguished by the composition of their cones in α- and β-selinene, alloaromadendrene, β- and γ-elemene or α-bergamotene. This model also revealed that the bitterness of beers was imparted by acylphloroglucinols derivatives **(Figure 5C)**, and then corroborated the bitter potential of commercial varieties compared to wild hops, which were not distributed along the axes of the bitterness **(Figure 4A)**.

4. Discussion

This study was focused on the chemical composition of wild hops and their aromatic potential in beers **(Figure 6)**. As regards the chemistry of cones, we identified the main volatile compounds of hops, *α*-humulene, *β*-myrcene and *β*-caryophyllene, in all accessions of our collection. In particular, *α*-humulene and *β*-caryophyllene are known for their spicy and woody smell (Rettberg et al., 2018). We also found original volatile compounds, present in a few wild accessions, as *α*- and *β*-selinene, alloaromadendrene or *β*- and *γ*-elemene. Patzak et al. (2010) reported that selinene was particularly present in wild hops compared with North American hops. This work also highlighted the strong chemical opposition between commercial and old varieties compared with wild accessions on their phytochemical composition (volatile compounds and phenolic compounds) and gustatory quality. A certain form of opposition between varieties and experimental germplasms had already been reported by Yan et al. (2019) especially on volatile compound composition, using GC–accTOFMS, on 30 Australian hop genotypes. Furthermore, recent studies such as Morcol et al., (2020) or Van Holle et al. (2021) suggested a terroir effect on hop chemical composition. The results of this study also served this hypothesis as chemical diversity underlined by non-targeted analyses was much reduced on the accessions of this study, all coming from the same experimental hop field, compared to the accessions collected *in-situ* in our previous study Paguet et al. (2023). Nevertheless, the *ex-situ* collection also allowed a monitoring of hop maturation and thus collection of the cones at their optimum maturity, which avoided the appearance of degradation compounds. For instance, contrary to our previous study, we noticed high levels of *β*-myrcene in all accessions without exception. Myrcene usually does not make a contribution to hop aroma in beer, because its concentration is often far below the sensory threshold level, due to its evaporation during wort boiling Kishimoto et al. (2005). However, it may be used as a marker for cones ripening because monoterpenes are the last produced metabolites (Briggs et al., 2004). Some specific hop volatile components, such as geraniol for Cascade or *β*-pinene for Centennial, were identified as statistically relevant for forecasting dry-hop aroma quality (Lafontaine et al., 2018). Phytochemical profiling of our collection led to the identification of some wild accessions with a profile close to those of commercial cultivars (e.g. accessions I3 and G2). Previous genotyping in Paguet et al. (2023) revealed that wild accessions of our collection were significantly different from commercial and old cultivars grown in the north of France and were therefore not from an existing commercial variety **(Figure S5)**. Hence, the chemical diversity of wild accessions, even under *ex-situ* conditions, was probably due to the wide genetic diversity previously observed and is also reflected in significant morphological differences **(Figure S6)**. Among this chemical diversity, we have identified some derivatives of bitter acids. Wild accessions revealed high levels in *β*-acids. *β*-acids do not directly impact the taste of beer, but their oxidation in hulupones provides a very bitter taste to the beer (Van Cleemput et al., 2009).

Hence, after highlighting the chemical diversity of these hops grown under the same conditions, we evaluated their aromatic potential after brewing. An intra-batch physico-chemical characterisation made it possible to check that all the panellists tasted the same product. Nevertheless, inter-batch variations were observed, particularly in terms of the product's acidity and its residual sugar content. These differences may result from slightly different fermentation conditions, and did not really impact the descriptors appreciated by the panellists and therefore should not have significantly affected the taste characterisation of the products. After evaluation by a panel, we underlined that some wild accessions had a gustative potential fairly close to commercial varieties, as the accession H1, with a bitter perception. Some other wild accessions, H2, I9 and K5, belonging to the same cluster on the dendrogram, rather showed hoppy and fruity taste as the commercial variety Cascade or the old variety Groene Bel. This fruity and hoppy taste may be due to the presence of some volatile compounds, as *γ*-elemene or *α*- or *β*-selinene, which were also detected after brewing by SBSE and were known for their herbal and fresh flavors **(Table S1)**. These statements have been confirmed by the OPLS model, which underlined the influence of acylphloroglucinol derivatives and main hop volatile compounds on the bitterness and aromatic properties of beers brewed with commercial varieties on one hand, and the influence of less common volatile compounds and typical of our wild hop collection, on the other. However, the aromatic impact of hops in beer remains very complex to study because

beer is a complex matrix due to the interaction with the other ingredients of the beer and the effects of fermentation etc. (Dietz et al., 2020), as evidenced by the relative low validation score of our OPLS model. For example, the presence of citronellol, found in some beer composition but in no single hop**,** can be derived from a *de novo* synthesis, induced by yeast during the fermentation (Dietz et al., 2020). Hence, Van Holle et al. (2017) chose to carry out a sensory analysis on single hop beers in order to enhance the perception of the aromatic contribution of tested hop on beer. Machado et al., (2021) studied wild accessions coming from Portugal and assessed their gustative potential on dry-hopped beers. Dry-hopping allowed highlighting of the aromatic variations between different accessions (Podeszwa & Harasym, 2016). In our study, we also greatly reduced bias because we tested our hops in a same lager-style recipe, with a unique magnum bittering hop to provide bitterness, and in steeping (whirlpool) hop mode. The only ingredient modifying the recipe was the aromatic hops, wild or commercial, to be tested and it was added after boiling in infusion to preserve the volatile compounds. However, it could also be interesting to test in parallel the organoleptic profile of hops, without brewing, to avoid the interaction with malt and fermentation metabolites, as Martins et al. (2020) evaluated the aromatic potential of hops by check-all-that-apply analysis.

5. Conclusion

By means of a targeted and untargeted chemical fingerprinting and chemometrics approach, this study was able to identify compounds in our wild hops collection from Northern France that make them distinct from commercial varieties. Volatile compound analysis revealed a strong opposition between commercial and old cultivars and wild accessions of our collection; this opposition was also noticeable on the quantification of phenolic compounds and sensory evaluation, but less strong as regards untargeted metabolomics.

Some hops of wild accessions, such as I3 and G2, showed a chemical composition fairly close to commercial varieties. However, other wild accessions, such as H2, K5 and I9, showed an aromatic potential more pronounced during sensory analysis and close to the commercial variety Cascade or the old variety Groene Bel. OPLS-DA allowed the identification of chemical markers of cones which lead to a beer with sensory characteristics close to those brewed with the cultivars currently used by brewers. This study underlined the potential for further investigating genetic markers to understand the differences in the volatile compound concentrations as the phenotypic expression for each hop cultivar. These results illustrated the difference between commercially germplasms resulting from robust selection programs and the wild germplasms. This analytical approach thereby confirmed the potential of wild hops for varietal development and gave some information to implement a systematic targeted breeding approach.

Fundings

This work has been carried out in the framework of Feader project 2021-2024 (n° 35/2020) which is financed by European Union and the Hauts-de-France Region, as well as the CPER BiHauts Eco de France funded by both the French State and the Hauts-de-France Region. The authors gratefully thank the Hauts-de-France Region and European Union (Feder), the University of Lille (I-Site) and the “Fondation de la brasserie et de la malterie française” for the funding of the PhD fellow Anne-Sophie Paguet.

Acknowledgments

The authors acknowledge Fabrice Henry, the director of the Douai High School for hosting the experimental hop field. We also thank all the members of the tasting panel and Lydia Lefki for her help in sensory analyses. We acknowledge The FREDON Hauts-de-France for monitoring pathogens and pests, as well as Aurélien Honoré and Anne-Hélène Martin to the Chambre d’Agriculture de la Région

Hauts-de-France. We thank sincerely Dominique Huges for English grammar corrections.

Author's contribution

ASP: Investigation; Methodology; Formal analysis; Writing – original draft; **AS:** Project administration; Supervision; Writing-review & editing; **GL:** Conceptualization; Investigation in particular of the harvesting and monitoring of hop field; Writing-review & editing; **MV, SC:** Supervision of the sensory analysis; Methodology; Formal analysis; Writing-review & editing; **DL, ND:** Investigation in particular of brewing; **JS, FM, CD:** Investigation in particular of the chemistry part; **DM:** Investigation and Methodology in particular of High resolution mass spectrometry part; **MLF:** Supervision of GC-MS analysis; Methodology**;** Writing-review & editing; **RM, JXF:** Supervision of untargeted metabolomic analysis; Methodology; Formal analysis; Writing-review & editing; **SS:** Project administration; Writing-review & editing; **CR:** Conceptualization; Investigation and Supervision in particular of the harvesting and chemistry part, as well as the overall approach of the project; Methodology; Validation; Project administration; Funding acquisition; Writing – original draft and review & editing.

References

American Society of Brewing Chemists. (1992). Methods of Analysis. *Hops Methods*, 1–2.

Asano, K., & Hashimoto, N. (1980). Isolation and Characterization of Foaming Proteins of Beer. *American Society of Brewing Chemists*, *38*(4), 129–136.

Bart-Haas Group. (2021). *Bart-Haas report*.

Bocquet, L., Sahpaz, S., Bonneau, N., Beaufay, C., Mahieux, S., Samaillie, J., Roumy, V., Jacquin, J., Bordage, S., Hennebelle, T., Chai, F., Quetin-Leclercq, J., Neut, C., & Rivière, C. (2019). Phenolic Compounds from Humulus lupulus as Natural Antimicrobial Products: New Weapons in the Fight against Methicillin Resistant Staphylococcus aureus, Leishmania mexicana and Trypanosoma brucei Strains. *Molecules*, *24*(6), 1024. https://doi.org/10.3390/molecules24061024

Bocquet, L., Sahpaz, S., Hilbert, J. L., Rambaud, C., & Rivière, C. (2018). Humulus lupulus L., a very popular beer ingredient and medicinal plant: Overview of its phytochemistry, its bioactivity, and its biotechnology. *Phytochemistry Reviews*, *17*(5), 1047–1090. https://doi.org/10.1007/s11101-018-9584-y

Briggs, D., Boulton, C., Brookes, P., & Stevens, R. (2004). *Brewing: Science and Practice*. CRC Press. https://doi.org/10.1201/9780203024195

Bylesjö, M., Rantalaien, M., Cloarex, O., Nicholson, J. K., Holmes Elaine, & Trygg, J. (2007). OPLS discriminant analysis: Combining the strengths of PLS-DA and SIMCA classification. *Journal of Chemometrics*, *20*(8–10), 341–351.

De Keukeleire, D., Vindevogel, J., Szücs, R., & Sandra, P. (1992). The history and analytical chemistry of beer bitter acids. *TrAC Trends in Analytical Chemistry*, *11*(8), 275–280. https://doi.org/10.1016/0165-9936(92)87089-3

Dietz, C., Cook, D., Huismann, M., Wilson, C., & Ford, R. (2020). The multisensory perception of hop essential oil: A review. *Journal- Institute of Brewing*, *126*, 320–342. https://doi.org/10.1002/jib.622

Ducloux, A., Hédiard, L., & Vallez, T. (1910). *Les productions agricoles du Nord de la France.* (J.B. Baillière et fils, éditeurs).

Farag, M. A., Porzel, A., Schmidt, J., & Wessjohann, L. A. (2012). Metabolite profiling and fingerprinting of commercial cultivars of Humulus lupulus L. (hop): A comparison of MS and NMR methods in metabolomics. *Metabolomics*, *8*(3), 492–507. https://doi.org/10.1007/s11306-011-0335-y

Hong, K., Xu, Z., Wang, L., Johnpaul, A., Cheng, Y., Lv, C., & Ma, C. (2022). Varietal differences in the phytochemical components' accumulation and aroma profile of three Humulus lupulus cultivars. *Food Control*, *132*, 108499. https://doi.org/10.1016/j.foodcont.2021.108499

ICH. (2005). *International Conference on Harmonization of Technical Requirements forthe Registration of Pharmaceuticals for Human Use (ICH), Guideline Q2(R1)-Validation of Analytical Procedures: Text and Methodology*.

ISO. (2016). Analyse sensorielle—Méthodologie—Directives générales pour l'établissement d'un profil sensoriel. *ISO*. https://www.iso.org/cms/render/live/fr/sites/isoorg/contents/data/standard/05/80/58042.html

Kawa-Rygielska, J., Adamenko, K., Kucharska, A. Z., Prorok, P., & Piórecki, N. (2019). Physicochemical and antioxidative properties of Cornelian cherry beer. *Food Chemistry*, *281*, 147–153. https://doi.org/10.1016/j.foodchem.2018.12.093

Kishimoto, T., Wanikawa, A., Kagami, N., & Kawatsura, K. (2005). Analysis of Hop-Derived Terpenoids in Beer

and Evaluation of Their Behavior Using the Stir Bar−Sorptive Extraction Method with GC-MS. *Journal of Agricultural and Food Chemistry*, *53*(12), 4701–4707.

Lafontaine, S. R., Vollmer, D. M., Shellhammer, T. H., & Pereira, C. B. (2018). The Effectiveness of Hop Volatile Markers for Forecasting Dry-hop Aroma Intensity and Quality of Cascade and Centennial Hops. *BrewingScience*, *Volume 71*, 116–140. https://doi.org/10.23763/BrSc18-19lafontaine

Machado, J. C., Lehnhardt, F., Martins, Z. E., Faria, M. A., Kollmannsberger, H., Gastl, M., Becker, T., & Ferreira, I. M. P. L. V. O. (2021). Sensory and Olfactometry Chemometrics as Valuable Tools for Assessing Hops' Aroma Impact on Dry-Hopped Beers: A Study with Wild Portuguese Genotypes. *Foods*, *10*(6), Article 6. https://doi.org/10.3390/foods10061397

Martins, Z. E., Machado, J. C., Cunha, S. C., Barata, A. M., & Ferreira, I. M. P. L. V. O. (2020). A chemometric approach to compare Portuguese native hops with worldwide commercial varieties. *Journal of Chemometrics*. https://doi.org/10.1002/cem.3285

Morcol, T., Negrin, A., Matthews, P., & Kennelly, E. (2020). Hop (Humulus lupulus L.) terroir has large effect on a glycosylated green leaf volatile but not on other aroma glycosides. *Food Chemistry*, *321*, 126644. https://doi.org/10.1016/j.foodchem.2020.126644

Nicácio, K. de J., Ferreira, M. S., Katchborian-Neto, A., Costa, M. L., Murgu, M., Dias, D. F., Soares, M. G., & Chagas-Paula, D. A. (2022). Anti-Inflammatory Markers of Hops Cultivars (Humulus lupulus L.) Evaluated by Untargeted Metabolomics Strategy. *Chemistry & Biodiversity*, *19*(4), e202100966. https://doi.org/10.1002/cbdv.202100966

Paguet, A. S., Siah, A., Lefèvre, G., Sahpaz, S., & Rivière, C. (2022). Agronomic, genetic and chemical tools for hop cultivation and breeding. *Phytochemistry Reviews*, *21*(2), 667–708. https://doi.org/10.1007/s11101-022-09813-4

Paguet, A.-S., Siah, A., Lefèvre, G., Moureu, S., Cadalen, T., Samaillie, J., Michels, F., Deracinois, B., Flahaut, C., Alves Dos Santos, H., Etienne-Debaecker, A., Rambaud, C., Chollet, S., Molinié, R., Fontaine, J.-X., Waterlot, C., Fauconnier, M.-L., Sahpaz, S., & Rivière, C. (2023). Multivariate analysis of chemical and genetic diversity of wild Humulus lupulus L. (hop) collected in situ in northern France. *Phytochemistry*, *205*, 113508. https://doi.org/10.1016/j.phytochem.2022.113508

Patzak, J., Nesvadba, V., Henychová, A., & Krofta, K. (2010). Assessment of the genetic diversity of wild hops (Humulus lupulus L.) in Europe using chemical and molecular analyses. *Biochemical Systematics and Ecology*, *38*(2), 136–145. https://doi.org/10.1016/j.bse.2009.12.023

Podeszwa, T., & Harasym, J. (2016). *New methods of hopping (dryhoppingà and their impact on sensory properties of beer*. *21*, 81–88.

Rettberg, N., Biendl, M., & Garbe, L.-A. (2018). Hop Aroma and Hoppy Beer Flavor: Chemical Backgrounds and Analytical Tools—A Review. *Journal of the American Society of Brewing Chemists*, *76*(1), 1–20. https://doi.org/10.1080/03610470.2017.1402574

Schönberger, C., & Kostelecky, T. (2011). 125th Anniversary Review: The Role of Hops in Brewing. *Journal of the Institute of Brewing*, *117*(3), 259–267. https://doi.org/10.1002/j.2050-0416.2011.tb00471.x

Sharpe, F. R., & Laws, D. R. J. (1981). The Essential Oil of Hops a Review. *Journal of the Institute of Brewing*, *87*(2), 96–107. https://doi.org/10.1002/j.2050-0416.1981.tb03996.x

Van Cleemput, M., Cattoor, K., De Bosscher, K., Haegeman, G., De Keukeleire, D., & Heyerick, A. (2009). Hop (Humulus lupulus)-Derived Bitter Acids as Multipotent Bioactive Compounds. *Journal of Natural Products*, *72*(6), 1220–1230. https://doi.org/10.1021/np800740m

Van Holle, A., Landschoot, A. V., Roldán-Ruiz, I., Naudts, D., & Keukeleire, D. D. (2017). The brewing value of

Amarillo hops (Humulus lupulus L.) grown in northwestern USA: A preliminary study of terroir significance. *Journal of the Institute of Brewing*, *123*(3), 312–318. https://doi.org/10.1002/jib.433

Van Holle, A., Muylle, H., Haesaert, G., Naudts, D., De Keukeleire, D., Roldan-Ruiz, I., & Van Landschoot, A. (2021). Relevance of hop terroir for beer flavour. *Journal of the Institute of Brewing*. https://doi.org/10.1002/jib.648

Van Opstaele, F., De Rouck, G., De Clippeleer, J., Aerts, G., & De Cooman, L. (2010). Analytical and Sensory Assessment of Hoppy Aroma and Bitterness of Conventionally Hopped and Advanced Hopped Pilsner Beers. *Journal of the Institute of Brewing*, *116*(4), 445–458. https://doi.org/10.1002/j.2050-0416.2010.tb00796.x

Yan, D., Wong, Y. F., Shellie, R., Mariott, P., Whittock, S. P., & Koutoulis, A. (2019). Assessment of the phytochemical profiles of novel hop (Humulus lupulus L.) cultivars: A potential route to beer crafting. *Food Chemistry*, *275*, 15–23.

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

716

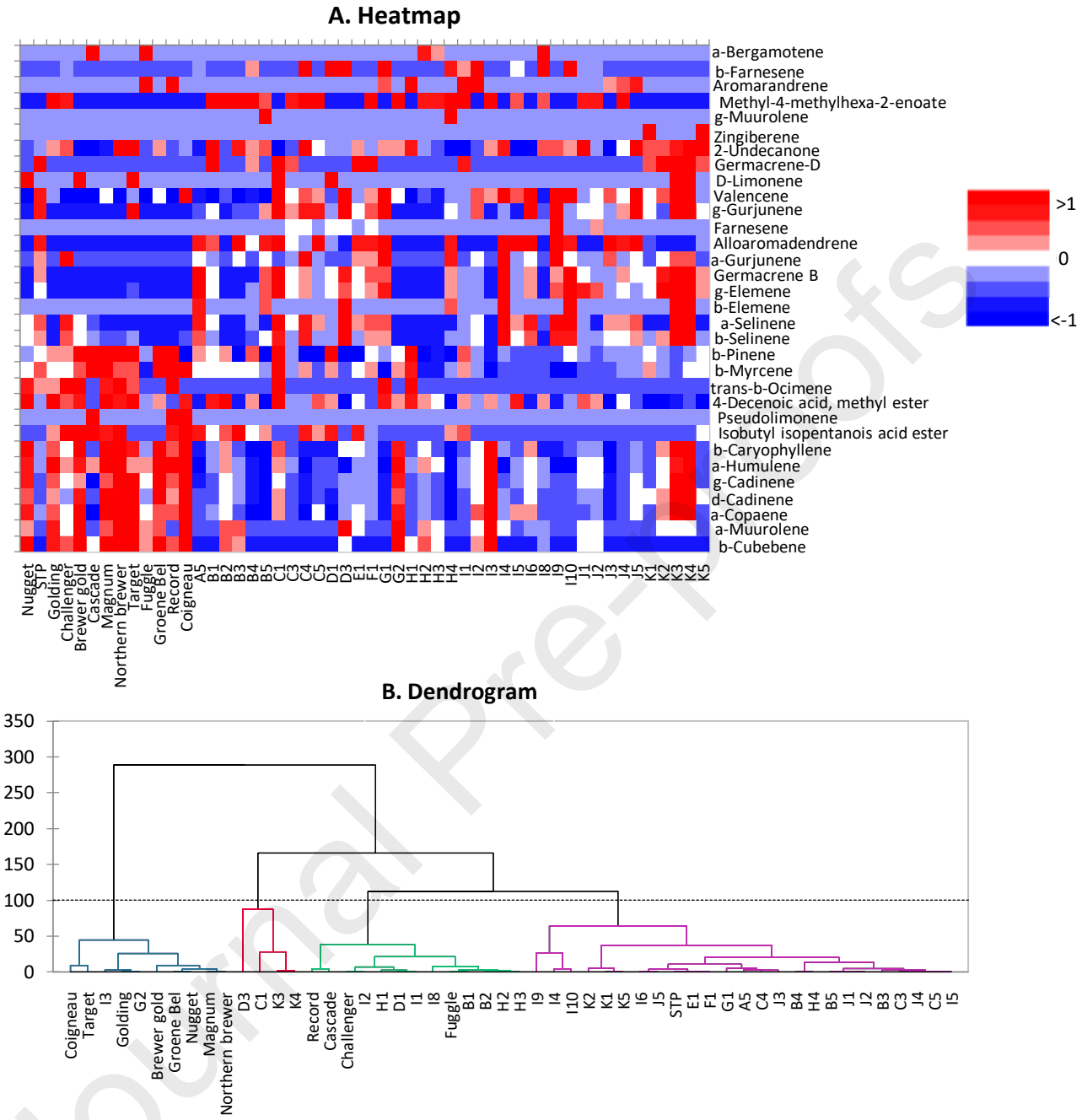
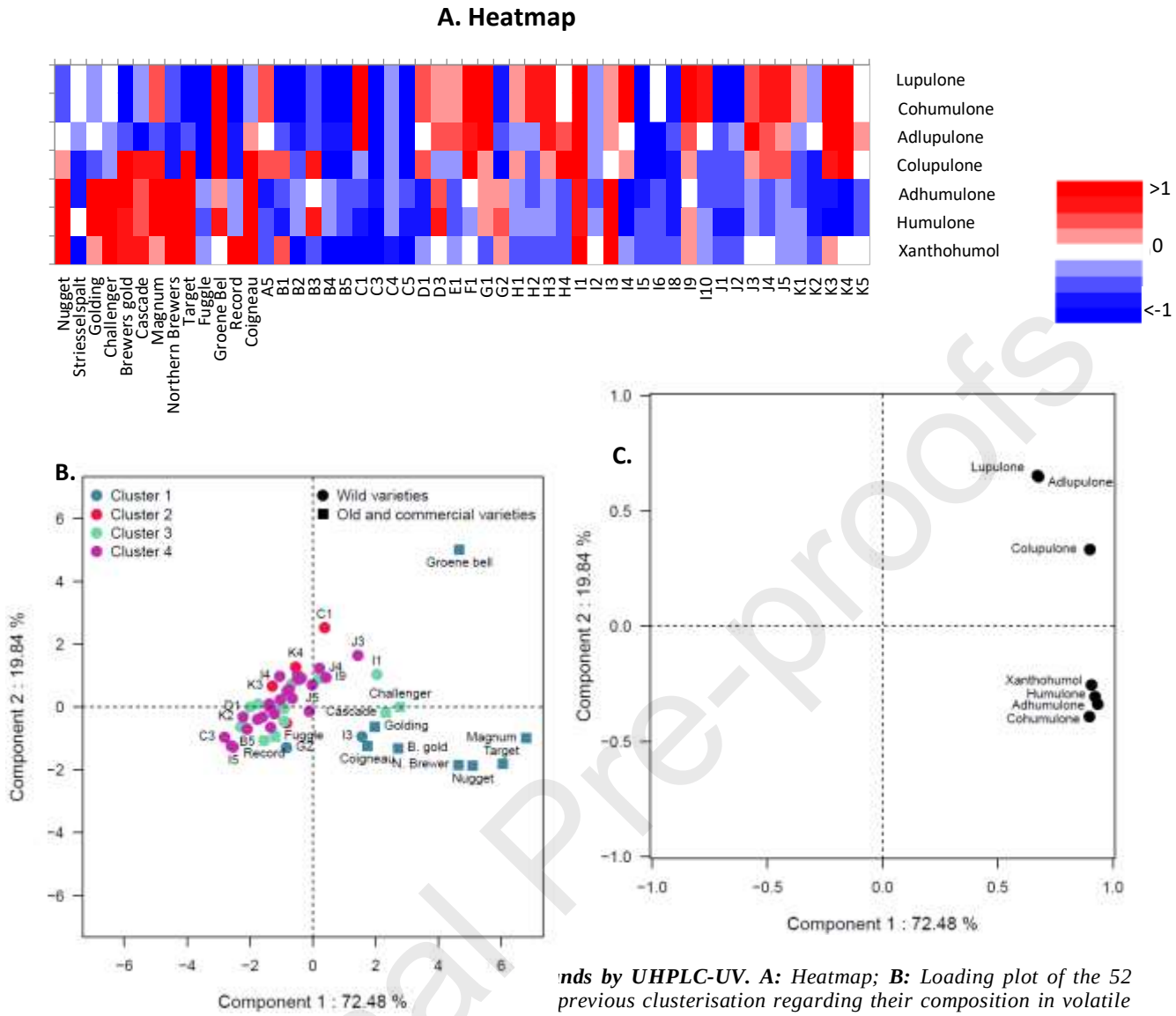


Figure 1. Volatile compound analysis by HS-SPME GC-MS based on the 32 compounds detected at least 1% in one accession. A. *Heatmap generated with normalized data for the top 32 compounds responsible for the differences between the profiles.*
B. *Dendrogram associated to the AHC (Ward's method, n=4). STP = Strisselspalt.*

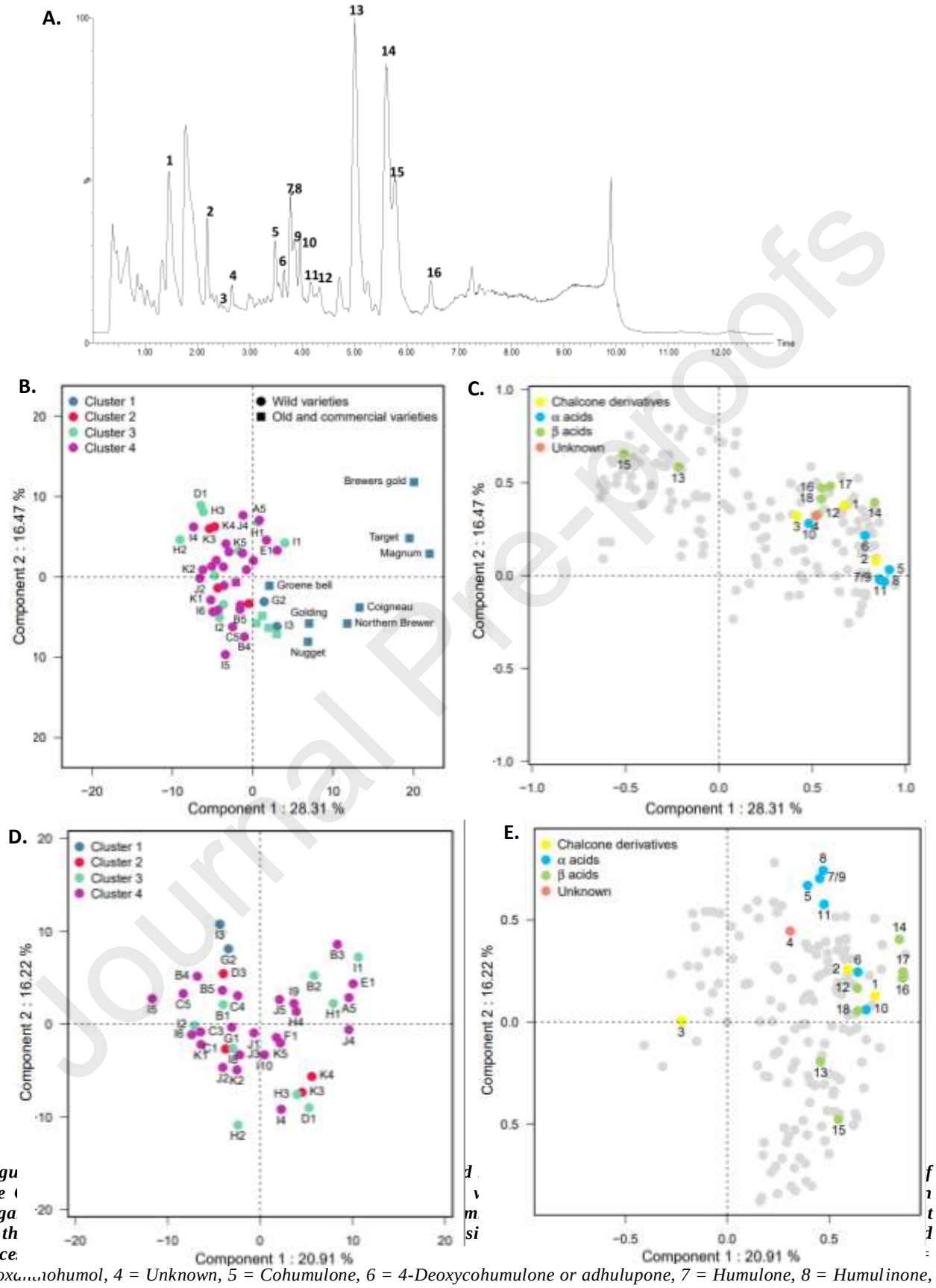
717



nds by UHPLC-UV. A: *Heatmap;* ***B:*** *Loading plot of the 52 previous clusterisation regarding their composition in volatile compounds, old and commercial cultivars represented by a square;* ***C:*** *Loading plot of the seven compounds quantified.*

718

719



Figu ***d*** ***f*** ***the*** ***v*** ***n*** ***rega*** ***m*** ***t*** ***of th*** ***si*** ***d*** ***acce***

Isoxanthohumol, 4 = Unknown, 5 = Cohumulone, 6 = 4-Deoxycohumulone or adhulupone, 7 = Humulone, 8 = Humulinone, 9 = Adhumulone, 10 = Deoxyhumulone, 11 = Prehumulone, 12 = Postlupulone, 13 = Colupulone, 14 = Lupulone, 15 = Adlupulone, 16 = Prelupulone.

720

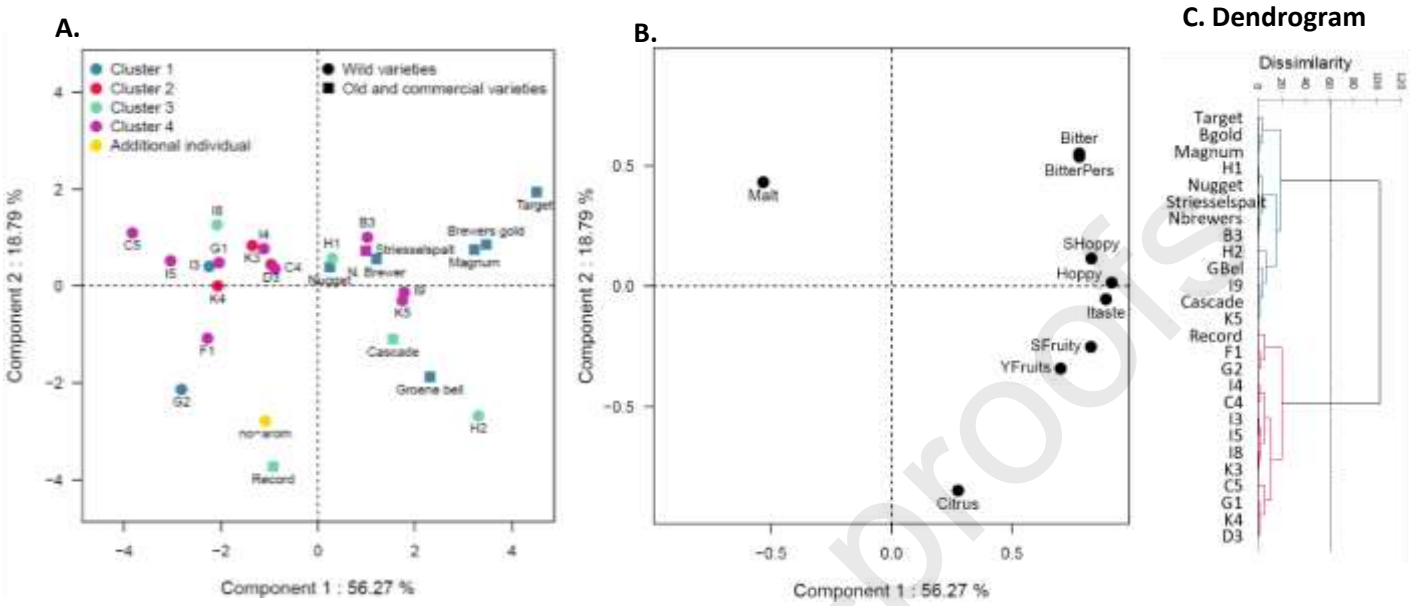


Figure 4. Analysis of the data panel. A**: Loading plot of the 27 beers on the dimensions 1 and 2 on the data of the sensory characterization by a panel. Individuals were coloured according to their composition in volatile compounds.* *The observation "no-arom" is brewed with no aromatic hop;* ***B: Loading plot of the sensorial descriptors used for the sensory analysis. *"SHoppy": hoppy smell; "Hoppy": hoppy taste; "Sfruity": fruity smell "YFruits": yellow fruits flavour; "Itaste": intensity of the taste; "Citrus": citrus flavour, "Malt": malt flavour; "Bitter": bitter flavour; "BitterPers": bitter persistence;* ***C: Dendrogram obtained by AHC plotting the data of 27 beers for the 9 descriptors (Ward's method, n=2).***

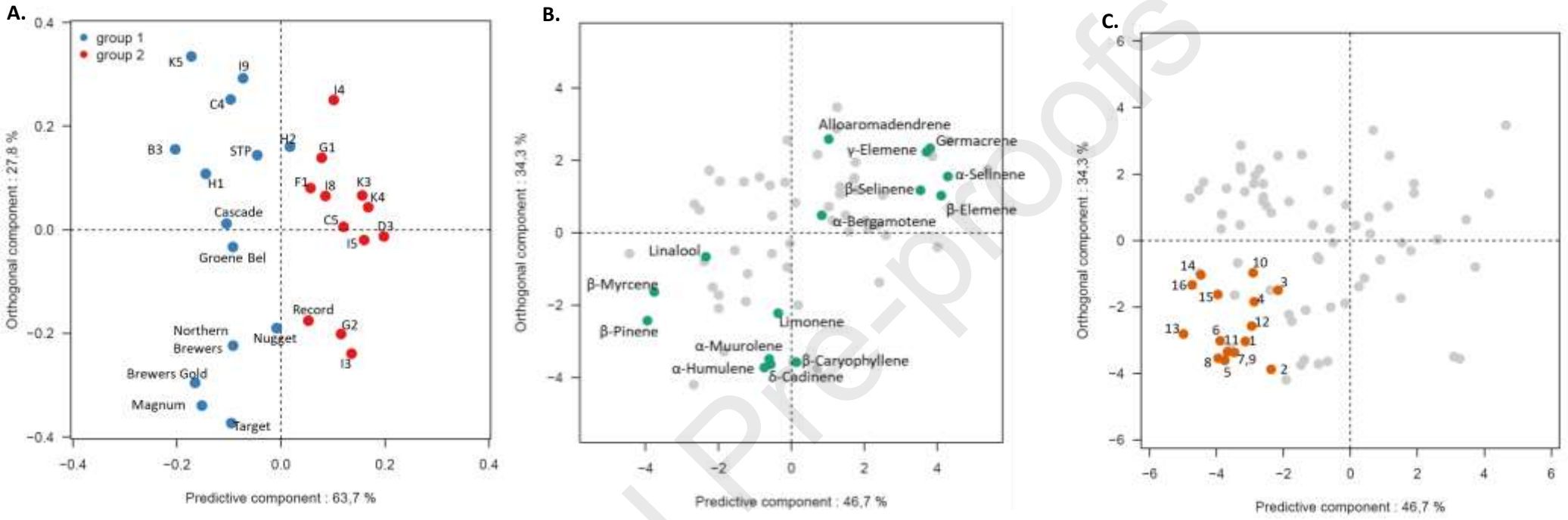


Figure 5. Multivariate association of beer metabolites with panel sensory traits. The association between chemical characterization by HS-SPME GC-MS (68 variables) and the UHPLC-ESI-Qtof (160 variables) was evaluated by OPLSA-DA using HCA clusters from sensory analysis. A*: score plot of the aromatic qualities of beers according the panel test;* ***B:*** *loading plot hop volatile compounds analyzed by HS-SPME GC-MS;* ***C:*** *loading plot untargeted metabolomics by UHPLC-HRMS-ESI-Qtof. 1 = Desmethylxanthohumol, 2 = Xanthohumol, 3 = Isoxanthohumol, 4 = Unkown, 5 = Cohumulone, 6 = 4-Deoxycohumulone or adhulupone, 7 = Humulone, 8 = Humulinone, 9 = Adhumulone, 10 = Deoxyhumulone, 11 = Prehumulone, 12 = Postlupulone, 13 = Colupulone, 14 = Lupulone, 15 = Adlupulone, 16 = Prelupulone.*

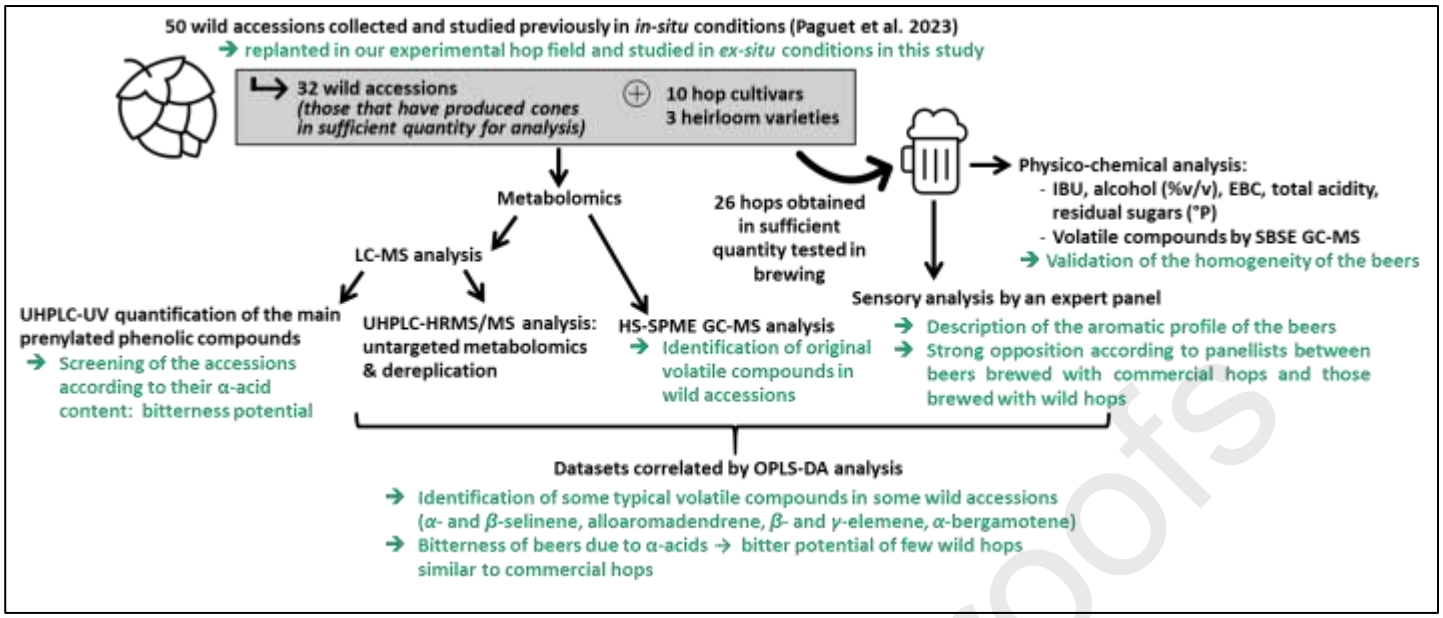


Figure 6. ***Summary diagram of the analyses carried out in this research and the main findings obtained.***

Highlights

- Few wild hops, such as I3, have a similar chemical compostion to commercial hops.
- Wild hops produce original volatile compounds compared with commercial hops.
- Some wild hops show an aromatic potential after brewing.
- Discriminant analysis opposes beers brewed with wild hops to those with commercial hops.
- This observation is consistent with their chemical composition.