Research papers

Influence of the drying process of Cascade hop and the dry-hopping technique on the chemical, aromatic and sensory quality of the beer

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Abstract: Drying techniques are important for hop storage and quality. The stage of hop addition in beer is another important issue. This study focuses the impact of two drying techniques [freeze-dryer (F) and hot-stove (H)] of Cascade hop, on the chemical, aromatic and sensory quality of beer, comparing beers produced without (BF and BH) and with dry-hopping technique (BFDH and BHDH). Dry-hopping with H significantly increased the bitterness index and reduced the titratable acidity. Isoamyl acetate (450.60 µg/L) and ethyl caprylate (313.60 µg/L) were in high content especially in BH while, ethyl-*n*-caproate (359.37 µg/L) had the highest content in BF. The beers made with dry-hopping technique, had a significantly higher content in terpenes especially in BFDH (1006.18 µg/L). Sensory evaluation indicated difference preferences, with freeze-dried hop beers generally favored. In conclusion, depending on the type of beer desired, hops dried in different way and a specific hopping technique can be chosen.

Keywords: *Humulus lupulus* L; Dehydration techniques; Dry-hopping; VOCs profile; Beer sensory quality.

1. Introduction

Hop (*Humulus lupulus L.*) is an essential raw material for beer production as it provides an increased shelf-life, bitterness, and aroma to beer (Heřmánek et al., 2018; Raut et al., 2021; Steinhaus & Schieberle, 2000). The quality of the raw material is strongly influenced by the postharvest processing methodologies. In order to be marketed, as well as used in the beer production process, the raw material must undergo particular conditioning and processing methods. The water concentration inside the fresh inflorescences (moisture) is about 80 % (Monacci et al., 2023; Verzele & De Keukeleire, 1991) and, in order to be marketed and used in beer production, the raw material must undergo a drying process (Neve, 2012; Rybacek, 2012) to reduce the water content down to 8 - 11 % (Heřmánek et al., 2018; Rybka et al., 2018).

In this regard, it is important that the matrix undergoes a drying process in the shortest time, in order to preserve its quality and increase its shelf-life (Rossini et al., 2021). The temperature at which the drying process is conducted inevitably determines the loss of chemical compounds present within the inflorescence (Rybka et al., 2018). The quantity of essential oils presents in the fresh product can be reduced by 30-40% following processing, due to the extreme volatility of these compounds (Nance & Setzer, 2011; Rybka et al., 2018). For this reason, the temperature control which the process is carried out to, is of extreme importance with regard to the quality of the final beer production (Raut et al., 2020). Kiln and belt drying are used by the hop industry for commercial scale, where moisture is evaporated under a continuous flow of hot air stream (Ratti, 2001). Oven drying and dehydrator drying have similar physica principle. Ferenczi et al. (2018) reported the influence of alternative drying techniques (microwave vacuum, freeze and hot-air drying) on aroma retention in Centennial hops. More recently, Su et al. (2022) have compared freeze-drying, dehydrator-drying, and oven-drying of hops, studying the aroma profile, concluding that dehydrator-drying is the best method.

Another important factor in beer process, is the stage of hop addition. There is no doubt on the significant contribution of dry-hopping to the renaissance of craft beer and the subsequent boom in sales of craft beers around the world (Maye et al., 2018; Oladokun et al., 2017). Dry-hopping represents a relatively simple means of improving the beer flavor; brewers add between 2 – 12 g/L of hops in the form of cones or pellets into beer during fermentation or conditioning for periods ranging from several days to weeks (Hauser et al., 2019; Lafontaine & Shellhammer, 2019). The added hops can be left in beer with no mixing (static dry-hopping) or with mixing, i.e. using a mechanical pump or CO_2 gas (dynamic dry-hopping). Perhaps one of the unintended consequences of dry-hopping is the effect this process has on perceived beer bitterness. Several studies have shown an increase in both measured analytical and perceived bitterness in dry-hopped beers (Algazzali & Shellhammer, 2016; Lafontaine & Shellhammer, 2019; Maye et al., 2016). Brewers can also add hop oil essences to beer to provide specific flavor characters that mimic dry-hopped flavor (Gomes et al., 2022; Klimczak et al., 2023). The increase in aroma perceived in dry-hopped beers versus conventionally hopped beers is thought to be due to elevated levels of several volatile terpene compounds, hydrocarbons and their derivatives e.g. linalool, myrcene, humulene, β-citronellol and geraniol (Klimczak et al., 2023; Oladokun et al., 2017; Takoi et al., 2010). Since these chemicals are rapidly lost through evaporation during wort boiling or fermentation, they are seldom perceived in beers that are not dry-hopped (Lafontaine & Shellhammer, 2019). Furthermore, the presence of yeast during dry-hopping adds an extra level of complexity to this process; the biotransformation of certain hop volatile compounds during and post fermentation (maturation), e.g., in the conversion of geraniol into β-citronellol has been seen (Takoi et al., 2010). Moreover, the presence of yeast during dry-hopping, may metabolize dissolved oxygen during dry-hopping, thereby protecting beer and hop volatiles from oxidation during the process (Gomes et al., 2022; Oladokun et al., 2017).

Our hypothesis for this study was that the VOCs (volatile organic compounds), other chemical characteristics and sensory appreciation would be positively, and differently affected by the drying techniques (freeze-drying or hot-stove drying) and by the dry-hopping technique.

2. Materials and Methods

2.1 Raw material

The raw material, consisted in female inflorescences of hop (*Humulus lupulus* L.) cv. Cascade, supplied by Azienda Agricola Opificio Birrario (Crespina-Lorenzana, Pisa, Italy). The total quantity of inflorescences, following the removal of leaves, stems and foreign material, was 25 kg. Two different water content reduction techniques were used: (i) a freeze-dryer (F), in LyoQuest lyophilizer (Azbil Telstar, S.L.U., Terrassa, Spain) with two cycles, each one lasting for 24 hours, at the temperature of - 52.4 °C at a pressure of 0.072 mBar; (ii) a hot-stove (H), in Heratherm® OMS100 (Thermo Fisher Scientific, Milan, Italy) for two days in dry air at temperature of 40 °C.

For each water reduction technique, approx. 10 kg of fresh hop inflorescences were divided in 3 trays placed in the stove or in the freeze-dryer. In Table 1 we report the chemical parameters of hop after the drying treatment. Data are exspressed on the basis of the dry matter (dm), obtained at 105 °C in ventilated stove up to constant weight (Bianchi et al., 2022).

Table 1. Chemical parameters of hop after the 2 different drying treatments (F and H). Data are the mean (±SD) of 3 trays with hops.

Chemical parameters	Units	F	H
α-acid	(%)	7.17 ± 0.09	7.29 ± 0.08
β-acid	(%)	5.72 ± 0.05	6.73 ± 0.03*

HSI	-	0.18 ± 0.02	0.23 ± 0.01*
Total polyphenols	(g GAE/kg dm)	442.61 ± 3.31*	339.48 ± 1.03
ABTS	(mmol TE /kg dm)	34.42 ± 0.49*	22.32 ± 0.64
DPPH	(mmol TE/kg dm)	20.25 ± 0.51*	13.01 ± 0.25
Chlorophyll-a	(g/kg dm)	28.86 ± 0.44*	26.99 ± 0.28
Chlorophyll-b	(g/kg dm)	52.02 ± 0.63	55.71 ± 0.21*
Total Chlorophyll	(g/kg dm)	80.99 ± 0.61	82.60 ± 0.29*
Carotenoids	(g/kg dm)	53.02 ± 0.27	54.91 ± 0.41*
Essential oil yield	(% V/w)	4.99 ± 0.09*	4.64 ± 0.11

Asterisks refer to significant differences (Tukey, $p < 0.05$).

2.2 Beer production

The ingredients used for beer production were: 17 kg of malt Maris Otter (Muntons, Stowmarket, United Kingdom), 17 kg of malt Pilsner (Weyermann, Bamberg, Germany), 300 g of dried hop (F and H), 40 g of dry yeast SafAle™ US-05 (Fermentis, Marquette-lez-Lille, France) and water (85 L in mash, 35 L in sparge).

Beer was produced in spring 2023 in an Easy 100 stainless-steel plant (Polsinelli Enologia Srl, Isola del Liri, Italy) to produce 100 L of beer, following the steps reported below:

(i) Mash-in: insertion of ground malts at 45 ± 2 °C for 15 min.

(ii) Protein rest: 55 °C for 10 min.

(iii) β-amylase rest: 65 °C for 50 min.

(iv) α-amylase rest: 72 °C for 15 min.

(v) Mash-out: 78 °C for 5 min.

(vi) The wort was separated from trub using a steel filter Halo (Polsinelli Enologia Srl, Isola del Liri, Italy) placed on the bottom of the mash tank.

(vii) Sparge with water at 78 °C through the use of a stainless steel filter (Fly sparge, Polsinelli Enologia Srl, Isola del Liri, Italy) for the sugar extraction from trub.

(viii) The wort was divided into 2 tanks and boiled for 60 min at 100 ± 3 °C.

(ix) The hop was added in 3 portions using hop bags (100 g each) at 0 min, 30 min and 50 min from the beginning of boiling, to have different level of hop acids conversion. The dry products (300 g each) from 2 different techniques (freeze-dryer (F), hot-stove (H)) was used as aforedescribed. To facilitate the extraction and conversion of the hops, the wort was kept stirring during boiling, using an electrically driven propeller placed under the lid of the boiling tank.

(x) The beer wort was cooled (23 ± 2 °C) using a plate heat exchanger (Polsinelli Enologia Srl, Isola del Liri, Italy) and the yeast was inoculated (40 g/hL).

For the fermentation phase, the two beer-wort was divided into 2 (two each type) fermenters of 20 L:

1- Beer with freeze-dried hop added during the boiling phase (BF).

2- Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH).

3- Beer with hot-stove dried hop added during the boiling phase (BH).

4- Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

The primary fermentation lasted 11 days at 18 ± 2°C and on the 7th day of fermentation, a static dry-hopping for 48 hours (concentration of 5 g/L) has been done in the BFDH and BHDH beer-wort.

After the beer was manteinted for 5 days at 6 ± 1°C in cold room to allow the precipitation of the solid compounds (lagering), and was manually separated from the lees formed at the bottom of the fermenter trought the faucet.

Successively, the beer was bottled in 0.5 L glass bottles (about 20 bottles for each type) and capped with a crown cap. During the bottling phase, 6 g/L of commercial beet sugar were added to start the bottle secondary fermentation which lasted one month at 21 °C ± 2 °C and after the analyses were carried out.

2.3 Chemical characterization of hop

The chlorophyll (a and b), total chlorophyll and carotenoid contents were determined following the method reported by Lichtenthaler (1987). In particular, 5 grams of fresh sample (shredding using mortar) were used to perform a solid/liquid extraction (in triplicate) with 50 mL of 100% methanol, sonicating the mixture in an ultrasonic bath (Elma TI- H-15, Singen, Germany) operating at a frequency of 45 kHz for 30 min at 20 °C. The homogenate was centrifuged at 6869 *g* at 4 °C for 10 min in a refrigerated centrifuge (Thermofisher Scientific, Waltham, USA), and the supernatant solution was filtered (0.45 µm). The absorption of the supernatant was read at 665.2 nm ($A_{665.2}$), 652.4 nm ($A_{652.4}$), and 470.0 nm ($A_{652.4}$) in a spectrophotometer UV/Vis (Cary 60, Agilent Technologies, USA) againts the blank (100% methanol). Chlorophyll-a and chlorophyll-b, total chlorophyll, and carotenoids, expressing as [g/kg on dry matter (dm)], were estimated using the following equations:

$$\text{Chlorophyll}-\text{a}\ \ (\text{Ca}) \ = \ 16.72\ \ A_{665.2} - 9.16\ \ A_{652.4}$$

$$\text{Chlorophyll}-\text{b}\ \ (\text{Cb}) = \ 34.09\ \ A_{652.4} - 15.28\ \ A_{665.2}$$

$$\text{Total}\ \ \text{chlorophyll} = \ 1.44\ \ A_{665.2} + 24.93\ \ A_{652.4}$$

$$\text{Carotenoids}\ \ = \frac{1000A_{470} - 1.63\ \ \text{Ca} - 104.96\ \ \text{Cb}}{221}$$

The total polyphenols content and the anti-radical activity were determinated in an extract prepared using 80% methanol and 5 g of hops in a ratio 1:5 (solid/liquid extraction, w/v) and sonicated in an ultrasonic bath (45 kHz for 30 min at 25° C). The mixture was centrifuged (15 min, 6869 *g*) and the supernatant was filtered (0.45 µm).

The total polyphenols (g of gallic acid equivalents (GAE)/kg dm) was determined using the Folin–Ciocalteu colorimetric method recording the absorbance at 765 nm (Bianchi et al., 2023a) and the anti-radical activity (ABTS and DPPH free radical method) (mmol Trolox equivalents (TE)/kg dm) was determined spectrophotometrically at 734 nm and 515 nm respectively (Bianchi et al., 2023c), in comparison with a calibration curve of gallic acid (Polyphenols: 0–3 g/L) and Trolox (ABTS assay: 0.1–1.5 mM and DPPH assay: 0–200 µmol/L).

For the determination of α- and β-acids content in hop cones and the Hop Storage Index (HSI), 5 g of hops were shredding using mortar, placed in a beaker, and 100 mL of toluene was added. The sample was stirred in an ultrasonic bath (Elma TI- H-15, Singen, Germany) operating at a frequency of 45 kHz for 30 min at a temperature of 25 °C. After being transferred in a falcon tube and placed in a centrifuge at 6869 *g* for 10 min, the supernatant solution was filtered (0.45 µm). For the measurement, 0.5 mL supernatant was diluted with 9.5 mL of methanol (solution A). 1.5 mL of solution A was diluted with 23.5 mL of alkaline methanol (0.02 mL of 6 N sodium hydroxide in 100 mL of methanol) (solution B). The blank was prepared by diluting 0.5 mL of toluene following the same sequence of steps as for sample preparation. The reading was done by an UV/Vis spectrophotometer (Cary 60, Agilent Technologies, USA) in a 1 cm^3 quartz cuvette against blank, with detection of the absorbance of solution B at 355 nm, 325 nm, and 275 nm. The concentration of α- and β-acids (%) and the Hop Storage Index (HSI) was calculated according to the previously reported equations (Monacci et al., 2023).

The quantification of the essential oil yield of the hop samples was carried out in agreement with Van Simaeys et al. (2022). In particular, 10 g of hops (3 extractions were performed for each hop sample) and 1 L deionized water were placed in a 2 L glass flask to the hydro-distillation process for a total time of 2 hours. At the end, the volume (mL) of extracted hydrosol was quantified and the yield was calculated as reported in the following equation:

$$Yield\ \ essential\ \ oils\ \ (\%\ \ V/w) = \frac{Volume\ \ hydrosol\ \ (mL)}{sample\ \ weight\ \ (g)} \times 100$$

2.4 Chemical analysis of beer

Beer chemical analyses were carried out on four, 0.5 L bottles (4 bottles each beer production), and before analyses, the beers were degassed by ultrasound. Chemical parameters were carried out following the methods reported below:

- Alcohol (% V/V) was evaluated using a hydrostatic balance (Alcoweight M5, Exacta+Optech Labcenter SpA, Modena, Italy) on the density at 20 °C of the distillate, obtained by distillation (Dualstill, Exacta+Optech Labcenter SpA , Modena, Italy) of 100 mL of beer neutralized with $Ca(OH)_2$ (Nurgel et al., 2004).
- pH was determined using an XS pH 80 PRO Stirrer (XS Instrument, Carpi, Italy) (Attchelouwa et al., 2017).
- Residual sugars (g/L hexoses) were conducted using an enzyme kit (K-FRUGL, Megazyme Ltd., Wicklow, Ireland) (Mederios et al., 2023).
- Titratable acidity (g/L lactic acid) was assessed by acid/base titration of 25 mL sample with NaOH 0.1 N until reaching pH 8.3 (Attchelouwa et al., 2017).
- Volatile acidity (g/L lactic acid) was determined by NaOH (0.1 N) titration, using phenolphthalein as an indicator, on distillate obtained by steam distillation (Dualstill, Exacta+Optech Labcenter SpA, Modena, Italy) of 50 mL of beer (Zoecklein et al., 1990);
- Color according to the SRM (Standard Reference Method) scale is defined by its color through the reading of the absorbance at 420 nm multiplied by the factor 49.25 (Koren et al., 2020).
- Bitterness according to the IBU (International Bitterness Unit) scale was calculated by reading the absorbance at 275 nm multiplied by the factor 50, on the supernatant obtained after extraction of 10 mL of beer with 1 mL of HCl 3 N, 20 mL of isooctane, 50 µL of octanol (Martin et al., 2010).
- Total polyphenols (mg/L gallic acid) were determined using Folin–Ciocalteu colorimetric method (Mastrangelo et al., 2023).
- The anti-radical activity of beer (mmol Trolox equivalents (TE)/L) was determined by DPPH and ABTS free radical methods (Mastrangelo et al., 2023).

2.5 Volatile organic compounds (VOCs)

The analysis of VOCs of beer was performed as previously reported (Mastrangelo et al., 2023). In particular, 10 mL sample of beer (2 bottles each beer production), degassed by ultrasound, and 100 µL of a 2-octanol solution at 500 mg/L was added as an internal standard. The sample was deposited on a Hypersep Retain Prep (Thermo Fisher Scientific, Milan, Italy) cartridge (60 mg), activated with 2 mL dichloromethane, 2 mL methanol and 2 mL water. The analytes were eluted with 5mL of dichloromethane, collected in sovirel on the bottom of which 2 grams of anhydrous sodium sulfate had been inserted and placed in the freezer overnight. Samples were filtered with a cellulose filter to remove sodium sulfate and concentrated to a final volume of 200 µL under a stream of N_2. The GC apparatus consists of a Trace GC ultra-gas chromatograph with a Trace DSQ with quadrupole mass detector (Thermo Fisher Scientific, Milan, Italy) and a Stabilwax DA capillary column (Restek, Bellefonte, PA, USA; 30 m, 0.25mm i.d., and 0.25 µm film thickness) and He (constant flow of 1.0 mL/min) was used as The carrier gas.

GC temperature ramp was programmed as reported by Castro-Marín et al. (2018): from 45°C (maintained for 1 minute) to 100°C (maintained for 1 minute) at 3°C/min, then to 240°C (maintained for 10 minutes) at 5°C/min. The injection was performed at 250°C in spitless mode and the volume injected is 1 µL. Analyses were done in quadruplicate (four bottles) and GC-MS parameters were obtained by using Xcalibur v 4.1 software (Thermo Fisher Scientific, Milan, Italy). Identification and the quantification of compounds (µg/L) were carried out as previously reported (Mastrangelo et al., 2023).

Odor description was obtained from FLAVORNET (https://www.flavornet.org/index.html) and The Good Scents Company Information System (https://www.thegoodscentscompany.com). The odor thresholds was obtained from Odor Thresholds Detection & References (http://www.leffingwell.com/odorthre.htm) and the cited literature as reported in Table 3.

2.6 Sensory analysis

The beer sensory profile was evaluated by a panel of ten beer experts using QDA (qualitative discriminant analysis) method (Stone et al., 2004) at the SensoryLab of DiSTAS of Università Cattolica del Sacro Cuore compliant with the International Organization for Standardization standard (ISO 8589:2007) (Donadini et al., 2013). The samples were served randomly during the test with a rotated plan. The sensory scoresheet presented five main classes of parameters selected and slightly modified from Donaldson et al. (2012) and Donadini et al. (2013): visual, olfactory, tactile, gustatory and retro-olfactory perception. The first class was divided into: foam stability, foam compactness, turbidity, yellow straw color, golden yellow color, amber yellow color, and color pleasantness. The second class was divided into: olfactory intensity, olfactory complexity, floral, fruity, vegetable, malty and olfactory pleasantness. The third class was divided into: effervescence, body, sweet, sour, bitter, astringency, softness and balance. The last class was divided into flowers, vegetables, fruits, citrus fruits (orange, lemon, tangerin and grapefruit), tropical fruits (pineapple, banana, mango, kiwi and lychee), peach/apricot, apple/pear, yeast, malt, caramel, spicy, toasted, persistence, DMS-defects, mold-defects, cardboard-defects and retro-olfactory pleasantness. For all the samples, after the objective evaluation, the overall liking of the sample was evaluated. The intensity of each attribute was evaluated on a nine-point horizontally oriented scale anchored as ''not perceived at all'' and ''extremely intense''. Before starting the analysis, panel was calibrated around the median ± 1, calculated for each attribute, after the evaluation of an extra sample served as calibration sample. Repeatability, discrimination and collimation of the panel and panelists were evaluated thanks to the presence of an analytical replicate in the set (Vezzulli et al., 2021).

Ethical approval for the involvement of human subjects was granted by the Ethics Committee of of the University of Pisa (protocol n. 0088081/2023) and all the tasters were completely voluntary; informed consent was also obtained from each taster according to the ethical guidelines.

2.7 Statistical analysis

One-way ANOVA was run (CoStat, Version 6.451, CoHort Software, Pacific Grove, CA, USA) and Tukey's honestly significant difference (HSD) test with $p < 0.05$ for multiple comparison, was used for the chemical parameters.

The JMP 17 software was used to perform a principal component analysis (PCA) and a hierarchical cluster analysis (HCA) on the VOCs data as previously reported (Bianchi et al., 2023b).

Finally, sensory analysis results were processed by Big Sensory Soft 2.0 (version 2018) and elaborated in Microsoft Excel 2007, to be validated as the median values of the intensity scored by the panel to each sensory descriptor, then plotted as spider graphs.

3. Results and Discussion

As reported in Table 1 in M&M section, the dried hop chemical characteristics were different depending on the drying technique used. We adopted these dry products to produce the experimental beers, emphasizing the significant difference in polyphenols and antioxidant acitivties (higher in F technique) and β-acids (higher in H technique) and their role in beer.

3.1 Chemical characterization of beers

BH and BHDH samples had a significantly higher alcohol content than BF and BFDH samples (8.85% v/v in BH and 8.42% v/v in BHDH vs 6.34% v/v and 6.24% v/v in BF and BFDH, respectively) while no significant difference was found in residual sugar concentration (Table 2). The lower ethanol content in BF samples could be explained by a lower alcoholic fermentation rate and higher initial glyceropyruvic fermentation by yeasts; this hypothesis is validated by a higher volatile acidity especially in dry-hopping sample. This behaviour occurs generally speaking when we have a high sugar concentration in the matrix (formation of glycerol) (Bely et al., 2003; Mencarelli et al., 2021; Santini et al., 2023) or when competitive microorganisms occur (lactic bacteria or *non-Saccharomyces* yeasts) (Torres-Guardado et al., 2022) or some compounds are in higher concentration (i.e. polyphenols which can compete for oxygen) (Salmon, 2006) which halt or delay the beginning of alcoholic fermentation. Freeze-drying does not guarantee the complete removal of microorganisms (Yadav & Roopesh, 2020) and gram-positive bacteria (i.e. lactic bacteria) have a greater ability to survive than gram-negative ones in freeze-drying (Morgan & Vesey, 2009; Wang et al., 2023). Thus, the presence of these bacteria could have contributed to increase the volatile acidity and a higher content of polyphenols could have competed for oxygen. Even supposing an error in the wort division in the two tanks, however, before we poured the wort, we mixed it as usual in the division practice, and for this reason, we believe that the alcohol difference can only be due to metabolism.

Titratable acidity was higher in samples BH and BHDH while dry-hopping reduced acidity; the observed difference between BF and BH samples could be due to the degree of sterilization of the matrix. It is well documented in the literature that there is a pH increase using dry-hopping, and a consequent lowering of total acidity (Hauser et al., 2019; Lafontaine & Shellhammer, 2018; Rutnik et al., 2022).

Beer color is determined by the interaction between the different raw materials of the beer recipe, as well as by the temperatures which the mashing phase is conducted to, and by the potential enzymatic oxidation of polyphenols (Koren et al., 2020; Modesti et al., 2021; Pahl et al., 2016; Pettinelli et al., 2022). The color (SRM scale) of all beers values fell into the Amber category (Table 2), between 13 and 16. Beers produced using hot stove dried hops (BH: 13.92; BHDH: 13.32), had a lighter color than those produced by using freeze-dried hops (BF 16.09; BFDH 16.15). The cause might be a more marked oxidation of polyphenols in BF and BFDH samples, because the dried hops had a higher content in this class of compounds (Table 1). In fact, the color of beer is conditioned by the oxidation of monomers of the flavonols class and the oligomers of proanthocyanidins (Koren et al., 2020).

Table 2. Main chemical parameters of beers. Data are the mean (± SD) of 4 bottles. Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

Chemical parameters	Units	BF	BFDH	BH	BHDH
Alcohol	% V/V	6.37 ± 0.11 b	6.26 ± 0.14 b	8.85 ± 0.17 a	8.42 ± 0.12 a
pH	-	4.68 ± 0.02 b	4.84 ± 0.03 a	4.50 ± 0.03 c	4.63 ± 0.04 b
Residual sugars	g/L hexoses	0.47 ± 0.07 a	0.35 ± 0.06 a	0.39 ± 0.02 a	0.42 ± 0.03 a
Titratable acidity	g/L lactic acid	1.66 ± 0.15 b	1.23 ± 0.12 c	1.97 ± 0.13 a	1.77± 0.19 ab
Volatile acidity	g/L acetic acid	0.23 ± 0.02 b	0.41 ± 0.05 a	0.17 ± 0.04 bc	0.14 ± 0.03 c
Color (SRM)	-	16.09 ± 0.52 a	16.15 ± 0.62 a	13.92 ± 0.41 b	13.32 ± 0.42 b
IBU	-	10.43 ± 0.63 b	6.26 ± 0.51 d	8.93 ± 0.27 c	16.46 ± 0.58 a
Total polyphenols	mg/L gallic acid	395 ± 25 ab	421 ± 31 a	315 ± 9 c	352 ± 21 b
ABTS	mmol TE/L	1.34 ± 0.02 b	1.60 ± 0.05 a	1.26 ± 0.03 b	1.34 ± 0.04 b
DPPH	mmol TE/L	0.82 ± 0.03 b	0.96 ± 0.02 a	0.76 ± 0.04 b	0.81 ± 0.03 b

Different letters in each row refer to significant differences (Tukey, $p < 0.05$). n.d.= not detected.

In BHDH the dry-hopping technique significantly increased the bitterness index (IBU = 16.46) compared to BH (8.93) while the opposite was for BF and BFDH samples (Table 2). The value of this index is generally affected by the concentration of α-acids and polyphenols, and their oxidation with formation of humulinone (Bocquet et al., 2018; Hauser et al., 2019; Pahl et al., 2016; Rutnik et al., 2022; Salamon et al., 2022). It is unclear why we obtained the opposite trend by using dry-hopping in BF or in BH. It has been reported, in case of α-acids, it is known that factors, such as chemical composition of wort, its extract and pH, amount of hops added and α-acids' content, boiling time, and temperature at which hops were added influence the level of bitterness, and this phenomenon is further complicated when dry hopping is used. Due to the presence of humulinones, polyphenols, and α-acids, a relatively simple spectrophotometric determination of IBU can give erroneous results (Klimczak & Cioch-Skoneczny, 2023). Thus it is possible that data we had, are not completely correct. The concentration in α-acids in dried hops was similar while polyphenols were higher in F samples, and in beer BF, with or without dry-hopping, had the highest content. The use of dry hopping in BF reduced significantly the bitterness, notwithstanding a higher content in polyphenols than in BH and we discuss later a potential hypothesis. As aforementioned, total polyphenols and antioxidant power (ABTS and DDPH) was higher in BF and BFDH samples (Table 3) due the higher values in dried hops (Table 1). The quality of the freeze-dried matrix was also influencing the antioxidant power of the beer, especially following the dry-hopping technique. It is known that this technique determines a greater stability of the chemical-physical characteristics of the finished product over time, increasing its shelf-life (Bamforth, 2016). Moreover, through the techniques of late hopping and dry-hopping, the extraction of α-acids in solution is favored (Oladokun et al., 2017) with the result of greater stability against microbiological contamination (Michel et al., 2020).

3.2 VOCs profile in beers

As in all fermented beverages, apart from ethanol and carbon dioxide which are the main products of fermentation, there are also classes of VOCs in beer which are formed as secondary products of the main metabolism (respiration or fermentation) and other VOCs which resulted by secondary metabolism; all of them characterize beer quality. Olaniran et al. (2017) reported what they define as "Flavour-active volatile compounds in beer". Higher alcohols and esters represent the most important classes being the result of yeast fermentation. Their accurate discussion gives an idea of the preminent metabolism occurring during fermentation. Among the esters, 1/3 is represented by ethyl acetate (Jespersen & Jakobsen, 1996) which, if in low concentration, provides solvent nuance. Other classes in lower concentrations but important from an aromatic point of view, are terpenes, furans, ketones.

Table 3. Volatile Organic Compounds (VOCs) in beers and the related odor quality and odor threshold. Data are the mean (± SD) of 4 bottles. Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

VOCs (µg/L)	Odor quality	Odor threshold (µg/L)	BF	BFDH	BH	BHDH
Ethyl butyrate	citrus	0.76 (Olaniran et al., 2017)	71.75 ± 2.67 b	75.05 ± 2.11 b	88.85 ± 3.19 a	90.57 ± 5.87 a
Ethyl isovalerate	fruity	0.50 (Olaniran et al., 2017)	n.d.	n.d.	n.d.	4.18 ± 0.23 a
Isoamyl acetate	solvent	1200 (Olaniran et al., 2017)	361.63 ± 21.24 b	130.87 ± 11.46 c	450.60 ± 14.03 a	478.83 ± 21.52 a
Ethyl *n*-caproate	fruity	210 (Olaniran et al., 2017)	359.37 ± 9.73 a	164.22 ± 6.91 c	263.43 ± 13.56 b	265.91 ± 11.59 b
Ethyl heptanoate	fruity - strawberry	220 (Ma et al., 2020)	4.16 ± 0.89 c	7.39 ± 0.98 b	5.36 ± 0.57 c	10.08 ± 0.86 a
Ethyl caprylate	fruity - apple	900 (Olaniran et al., 2017)	258.30 ± 6.17 c	238.29 ± 3.01 d	313.60 ± 3.76 b	341.92 ± 8.16 a
Ethyl decanoate	floral	240 (Ma et al., 2020)	33.39 ± 2.02 c	42.58 ± 2.66 b	21.23 ± 3.51 d	55.26 ± 5.64 a
Ethyl benzoate	sweet- fruity	60 (Piornos et al., 2019)	0.32 ± 0.09 c	2.40 ± 0.35 a	1.52 ± 0.30 b	2.40 ± 0.26 a
Diethyl succinate	ethereal	6000 (Ma et al., 2020)	8.51 ± 0.49 b	5.81 ± 0.25 d	6.59 ± 0.60 c	9.66 ± 0.22 a
Ethyl 9-decenoate	green - waxy		9.97 ± 1.08 c	9.62 ± 1.58 c	17.86 ± 1.37 b	23.74 ± 2.83 a
1,3-Propanediol, diacetate	balsamic		21.29 ± 0.66 c	21.69 ± 0.75 c	26.65 ± 2.09 b	31.33 ± 1.48 a
Methyl salicylate	wintergreen	40 (Piornos et al., 2019)	7.49 ± 0.86 c	13.56 ± 1.32 a	5.01 ± 0.62 d	8.46 ± 0.72 b
Etil-4-idrossibutanoate	caramel		21.35 ± 2.33 c	28.82 ± 1.06 b	43.56 ± 2.54 a	46.71 ± 3.37 a
2-Feniletilacetate	flower - rose		128.63 ± 6.25 a	33.29 ± 2.35 c	90.74 ± 7.45 b	95.20 ± 5.06 b
Ethyl 2-hydroxy-3-phenylpropanoate	black pepper		8.83 ± 0.98 d	12.41 ± 1.03 c	21.00 ± 1.24 a	17.29 ± 1.79 b
Ethyl hydrogen succinate	ethereal		27.24 ± 1.30 a	11.66 ± 1.02 c	15.29 ± 2.08 b	12.27 ± 1.89 bc
Ethyl vanillate	vanilla	100 (Piornos et al., 2019)	n.d.	10.66 ± 1.15 b	7.07 ± 1.36 c	14.04 ± 1.23 a
Total Esters			**1322.23 ± 57.47 b**	**808.33 ± 38.41 c**	**1382.23 ± 58.57 b**	**1507.83 ± 73.19 a**

1-Propanol	alcohol	306000 (Peinado et al., 2004)	5.00 ± 0.63 c	5.45 ± 0.45 c	6.99 ± 0.62 b	11.67 ± 1.02 a
Isobutanol	sweet - alcohol	200000 (Olaniran et al., 2017)	212.83 ± 6.95 c	231.34 ± 4.06 b	228.02 ± 4.58 b	264.82 ± 10.36 a
trans-3-Penten-2-ol	fruity - green	400 (Piornos et al., 2019)	7.28 ± 1.52 a	n.d.	9.04 ± 1.44 a	n.d.
Isoamyl alcohol	alcohol	70000 (Olaniran et al., 2017)	4403.08 ± 35.24 c	4411.70 ± 46.49 c	4865.19 ± 89.77 a	4601.17 ± 32.35 b
1-Pentanol	alcohol	64000 (Peinado et al., 2004)	3.01 ± 2.02 a	4.97 ± 1.60 a	4.77 ± 1.98 a	4.98 ± 1.70 a
4-Methyl-2-pentanol	alcohol- pungent		14.19 ± 1.67 a	14.51 ± 1.21 a	15.18 ± 1.21 a	16.07 ± 1.93 a
2-Methyl-2-buten-1-ol	green - grass		4.79 ± 0.61 b	6.16 ± 0.95 a	4.82 ± 0.47 b	7.18 ± 0.52 a
3-Methyl-1-pentanol	whiskey		0.64 ± 0.25 b	0.99 ± 0.12 b	3.23 ± 0.68 a	3.68 ± 0.92 a
n-Hexanol	green - leafy	800 (Peinado et al., 2004)	16.40 ± 2.01 d	25.23 ± 1.11 b	20.03 ± 1.51 cd	31.38 ± 1.83 a
3-Ethoxy-1-propanol	fruity	100 (Peinado et al., 2004)	4.46 ± 0.84 b	4.62 ± 0.33 b	7.08 ± 0.33 a	7.87 ± 0.55 a
cis-3-Hexen-1-ol	green - leafy	400 (Ma et al., 2020)	6.91 ± 1.64 c	22.01 ± 1.56 a	4.37 ± 0.56 d	9.75 ± 0.82 b
1-Octen-3-ol	hearty - mushroom	20 (Ma et al., 2020)	1.23 ± 0.90 b	8.82 ± 1.80 a	n.d.	6.48 ± 1.03 a
1-Heptanol	alcohol	200 (Ma et al., 2020)	29.34 ± 1.97 b	31.19 ± 2.16 b	52.02 ± 3.16 a	53.22 ± 2.55 a
3-Methyl-2-octanol	spicy		2.57 ± 0.32 b	2.82 ± 0.24 ab	3.11 ± 0.28 a	3.26 ± 0.22 a
3-Nonanol	citrus		10.82 ± 0.86 ab	11.43 ± 1.08 a	9.59 ± 0.61 b	11.15 ± 1.00 a
2-Nonanol	citrus	50 (Piornos et al., 2019)	7.15 ± 1.53 a	6.83 ± 1.40 a	6.72 ± 1.04 a	7.32 ± 1.85 a
2-3-Butanediol	sweet - butter		341.27 ± 14.57 ab	315.57 ± 21.78 b	363.29 ± 11.78 a	361.10 ± 12.07 a
1-Ottanol	waxy	110 (Piornos et al., 2019)	12.06 ± 1.58 a	12.88 ± 2.11 a	11.90 ± 2.11 a	13.94 ± 1.54 a
Metionol	cooked vegetable		17.23 ± 0.69 a	16.44 ± 0.77 a	17.83 ± 0.77 a	17.82 ± 0.91 a
Benzyl alcohol	floral - almond	10000 (Piornos et al., 2019)	17.10 ± 2.10 c	72.53 ± 3.97 a	17.64 ± 4.97 c	33.52 ± 6.85 b
Phenethyl alcohol	floral - rose	3000 (Piornos et al., 2019)	7740.16 ± 164.27 b	7524.38 ± 135.22 b	8645.00 ± 124.08 a	7608.98 ± 122.35 b
Total Alcohols			**12857.54 ± 242.17 c**	**12729.86 ± 228.40 c**	**14295.81 ± 251.96 a**	**13075.33 ± 202.40 b**
Acetic acid	vinegar		123.59 ± 3.79 b	115.93 ± 2.11 c	133.36 ± 2.73 a	135.61 ± 1.98 a
Propanoic acid	rancid	420 (Peinado et al., 2004)	12.72 ± 1.24 b	16.95 ± 1.02 a	6.92 ± 1.11 d	9.11 ± 1.35 c
Isobutyric acid	butter	30000 (Peinado et al., 2004)	64.27 ± 3.74 b	58.76 ± 3.47 b	75.18 ± 4.28 a	79.57 ± 4.15 a
Butanoic acid	butter	2190 (Piornos et al., 2019)	24.02 ± 3.00 b	19.50 ± 4.72 b	26.45 ± 2.43 a	25.84 ± 2.81 a
Pentanoic acid	cheese - rancid	3000 (Peinado et al., 2004)	123.44 ± 7.76 b	108.74 ± 10.20 b	144.51 ± 9.38 a	155.00 ± 4.63 a
Hexanoic acid	fatty - cheese	3000 (Peinado et al., 2004)	1484.71 ± 35.48 a	1403.37 ± 82.21 a	1492.86 ± 47.56 a	1507.69 ± 122.08 a
(*E*)-2-Hexenoic acid	cheese - fatty		11.52 ± 2.66 c	31.76 ± 6.21 b	16.89 ± 3.01 c	45.08 ± 2.85 a
Caprylic acid	rancid - cheese	10000 (Peinado et al., 2004)	3309.91 ± 127.12 a	2810.37 ± 65.03 b	3136.37 ± 87.24 a	2810.10 ± 53.26 b
Nonanoic acid	rancid - cheese	1400 (Ma et al., 2020)	10.50 ± 1.26 b	12.02 ± 1.58 ab	14.23 ± 1.75 a	15.00 ± 1.23 a
Capric acid	rancid - cheese	6000 (Peinado et al., 2004)	883.68 ± 36.82 a	445.95 ± 20.19 bc	405.69 ± 21.61 c	489.13 ± 36.15 b
9-Decenoic acid	waxy		206.82 ± 9.94 b	124.55 ± 7.39 c	240.96 ± 14.84 a	210.22 ± 11.72 b

Benzoic acid	fruity		84.44 ± 4.25 bc	77.97 ± 5.26 c	115.88 ± 14.53 a	91.62 ± 7.66 b
Lauric Acid	soap	1000 (Ma et al., 2020)	180.00 ± 11.20 a	43.25 ± 6.24 c	81.18 ± 8.62 b	90.48 ± 7.00 b
Benzene acetic acid	honey	10000 (Piornos et al., 2019)	220.93 ± 13.98 a	142.98 ± 14.12 b	226.53 ± 21.75 a	165.74 ± 9.01 b
Phenyl propionic acid	floral		20.58 ± 2.49 bc	18.17 ± 1.13 c	30.41 ± 2.62 a	25.20 ± 1.83 b
Myristic acid	waxy	10000 (Piornos et al., 2019)	44.30 ± 2.08 a	14.65 ± 1.37 c	39.21 ± 3.66 a	27.23 ± 1.05 b
Palmitic acid	waxy	10000 (Piornos et al., 2019)	170.39 ± 10.73 b	99.05 ± 4.62 d	263.53 ± 15.82 a	140.55 ± 9.03 c
Stearic acid	waxy	20000 (Piornos et al., 2019)	49.44 ± 3.52 b	41.76 ± 3.74 c	118.70 ± 15.91 a	44.06 ± 4.53 bc
Total Acids			**7025.27 ± 281.05 a**	**5585.73 ± 240.62 b**	**6568.87 ± 278.84 a**	**6067.25 ± 282.32 b**
2,7-Dimethyl-2,6-octadiene	-		393.04 ± 19.48 a	418.71 ± 10.49 a	297.89 ± 16.26 b	321.51 ± 12.42 b
β-Myrcene	resinous - herbaceous	350 (Ong & Acree, 1999)	n.d.	68.95 ± 2.32 a	0.09 ± 0.04 b	45.14 ± 7.12 b
6-Methyl-5-hepten-2-one	green - citrus		0.95 ± 0.17 c	2.64 ± 0.19 b	1.46 ± 0.47 c	4.27 ± 0.97 a
cis-Linalool oxide	floral- earthy		0.96 ±0.18 c	4.16 ± 0.32 a	1.98 ± 0.44 b	4.11 ± 0.28 a
trans-Linalool oxide	floral- earthy		n.d.	1.06 ± 0.09 b	n.d.	1.98 ± 0.07 a
β-Linalool	floral - citrus	9 (Ong & Acree, 1999)	66.86 ± 1.99 c	137.51 ± 1.32 a	73.71 ± 2.76 b	140.26 ± 2.40 a
p-Menth-2-en-1-ol	fruity - herbal		0.22 ± 0.02 b	0.41 ± 0.12 a	0.21 ± 0.03 b	0.47 ± 0.09 a
β-Caryophyllene	cloves- turpentine	230 (Ong & Acree, 1999)	8.41 ± 0.77 c	16.85 ± 1.75 a	13.60 ± 0.92 ab	15.29 ± 0.78 a
Terpinene-4-ol	green-earthy		1.74 ± 0.44 b	4.19 ± 0.62 a	1.87 ± 0.66 b	3.62 ± 0.32 a
(*E*)-Farnesene epoxide	floral		10.84 ± 1.14 c	21.10 ± 1.90 b	12.05 ± 0.78 c	29.19 ± 2.10 a
α-Humulene	balsamic	450 (Ong & Acree, 1999)	2.36 ± 0.72 c	21.63 ± 1.89 a	3.14 ± 0.64 c	18.88 ± 1.15 b
α-Terpineol	citrus - woody	250 (Ma et al., 2020)	12.74 ± 0.73 b	27.22 ± 2.44 a	13.65 ± 0.78 b	29.09 ± 1.86 a
2,6-Dimethyl-1,5,7-octatrien-3-ol	camphor		2.05 ± 0.21 b	5.22 ± 0.82 a	2.56 ± 0.78 b	7.14 ± 1.64 a
β-Citronellol	floral - rose	25 (Ong & Acree, 1999)	20.75 ± 1.50 c	63.88 ± 7.48 a	19.37 ± 1.11 c	38.47 ± 3.21 b
cis-Geraniol	floral - rose	53 (Ong & Acree, 1999)	5.49 ± 0.70 d	14.45 ± 1.31 a	8.51 ± 0.34 c	10.95 ± 0.99 b
trans-Geraniol	floral - rose	1.1 (Ong & Acree, 1999)	0.30 ± 0.03 b	0.60 ± 0.11 a	0.60 ± 0.06 a	0.67 ± 0.04 a
p-Mentha-1,8-dien-7-ol	floral		16.01 ± 1.46 d	82.11 ± 1.46 a	37.56 ± 1.21 c	61.00 ± 2.54 b
8-Hydroxy linalool	fruity		10.33 ± 1.80 c	25.59 ± 1.98 a	13.59 ± 1.63 bc	16.50 ±1.25 b
Farnesol	floral - lily	20 (Piornos et al., 2019)	21.78 ± 1.09 b	22.87 ± 1.89 b	30.89 ± 2.26 a	29.00 ± 2.20 a
Dihydro-β-ionone	floral - violet		12.41 ± 2.86 c	67.02 ± 3.71 a	52.87 ± 3.01 b	54.90 ± 4.25 b
Total Terpenes			**587.23 ± 35.28 c**	**1006.18 ± 42.22 a**	**585.61 ± 29.28 c**	**832.44 ± 45.69 b**
1,1-Dimethyl-4-methylenehexahydro-1H-cyclopenta[c]furan	malty - caramel		n.d.	3.86 ± 0.35 b	n.d.	7.03 ± 0.64 a
Furfuryl alcohol	alcohol - ether		11.11 ± 0.70 ab	10.72 ± 0.46 b	12.19 ± 1.09 a	10.84 ± 0.64 b
5-Ethyldihydro-2(3H)-furanone	herbal - tobacco		2.77 ± 0.48 a	3.20 ± 0.23 a	3.23 ± 0.34 a	2.73 ± 0.45 a

2,3-Dihydro-5-hy-droxy-6-methyl-4H-pyran-4-one	malty - caramel		23.03 ± 1.11 a	21.00 ± 1.32 ab	20.50 ± 0.52 b	20.31 ± 0.93 b
2,3-Dihydro-benzofu-ran	fruity		31.43 ± 2.72 b	47.63 ±3.23 a	25.83 ± 2.07 c	31.36 ± 2.39 b
Total Furans			**68.34 ± 5.02 bc**	**86.41 ± 5.58 a**	**61.75 ± 4.02 c**	**72.27 ± 5.05 b**
2-Methoxy-4-vi-nylphenol	smoky - caramel	99 (Olaniran et al., 2017)	99.60 ± 5.17 b	108.26 ± 4.17 b	139.63 ± 2.25 a	143.52 ± 2.26 a
Acetovanillone	vanilla		33.81 ± 1.98 c	34.64 ± 1.18 c	42.86 ± 1.02 b	51.74 ± 3.25 a
Methoxy-eugenol	spicy	20 (Ma et al., 2020)	17.25 ± 0.95 c	20.09 ± 1.01 b	21.22 ± 1.02 ab	24.80 ± 2.28 a
trans-Cinnamic acid	floral - honey		72.39 ± 0.99 a	69.22 ± 2.07 a	63.99 ± 1.45 b	59.19 ± 2.41 b
Tyrosol	floral - fruity		155.29 ± 10.57 b	132.12 ± 9.09 c	208.95 ± 13.58 a	145.30 ± 11.56 bc
Total Phenols			**378.35 ± 19.66 c**	**364.34 ± 17.51 c**	**476.66 ± 19.33 a**	**424.55 ± 21.76 b**
Acetoin	butter	800 (Piornos et al., 2019)	45.84 ± 4.31 a	10.27 ± 1.24 c	15.47 ± 1.23 b	17.19 ± 1.20 b
3-Hydroxy-3-methyl-2-butanone	-		14.03 ± 1.01 a	12.07 ± 0.67 b	13.23 ± 0.50 ab	13.56 ± 0.58 a
γ-Nonalactone	coconut	30 (Piornos et al., 2019)	35.83 ± 0.73 b	36.42 ± 0.74 b	47.07 ± 1.22 a	45.13 ± 1.56 a
Total Ketones			**95.70 ± 6.33 a**	**58.75 ± 8.52 c**	**75.77 ± 5.21 b**	**75.89 ± 5.58 b**
n-Nonanal	rose - orange	2.8 (Ma et al., 2020)	2.20 ± 0.35 ab	1.97 ± 0.21 b	1.93 ±0.15 b	2.59 ± 0.24 a
Total Aldehydes			**2.20 ± 0.35 ab**	**1.97 ± 0.21 b**	**1.93 ± 0.15 b**	**2.59 ± 0.24 a**
2-Acetylpyrrole	bready - walnut	1700 (Piornos et al., 2019)	47.98 ± 1.82 a	40.24 ± 2.22 b	39.04 ± 2.82 b	40.46 ± 1.22 b
5-Methyl-1H-pyrrole-2-carboxaldehyde	roasted - cara-mel		7.15 ± 0.60 b	8.68 ± 0.35 a	7.45 ± 0.93 b	7.98 ± 0.39 ab
Styrene	-		n.d.	n.d.	14.68 ± 2.09 a	12.62 ± 1.86 a
Total Others			**55.13 ± 2.43 b**	**48.91 ± 2.57 c**	**61.18 ± 3.76 a**	**61.06 ± 3.47 a**

Different letters in each row refer to significant differences (Tukey, $p < 0.05$). n.d.= not detected.

Esters provide beer with fruity and floral scents (Brányik et al., 2008). In BF beers the concentration of esters was lower, and in BFDH, the lowest (Table 3). We found mainly isoamyl acetate, especially in BH samples (450.60 μg/L) and, very low concentration, in BFDH (130.87 μg/L); also ethyl caprylate was higher in BH samples while ethyl-n-caproate was the highest in BF but the lowest in BFDH (Table 3). Concentrations of isoamyl acetate above 0.2 mg/L give fruity scents (olfactory threshold 1.2 mg/L) (Olaniran et al., 2017), while ethyl caprylate and ethyl-n-caproate provide aromas of acidic apple in concentrations higher than 0.9 mg/L and 0.21 mg/L, respectively (Kobayashi et al., 2007) (Table 3). In our samples the detected content was below the olfactory perception threshold.

Alcohols were ten-fold higher than esters and the total highest concentration was in BH (Table 3). BF beers whatever was the dry-hopping treatment, had the lowest values. Among the higher alcohols, the most important in term of concentration was isoamyl alcohol (BH 4865.19 μg/L; BHDH 4601.17 μg/L; BF 4403.08 μg/L; BFDH 4411.70 μg/L) and 2-phenylethyl alcohol (BH 8645.00 μg/L; BHDH 7608.98 μg/L; BF 7740.16 μg/L; BFDH 7524.38 μg/L). Isoamyl alcohol if present in high concentration, negatively affects the drinkability of the product (Olaniran et al., 2017); however, the quantity detected in the test samples was below the limit at which this adverse effect is observed. 2-phenylethyl alcohol, which would give hints of rose, peppermint and orange blossom, was also below the threshold of perception (125 mg/L) (Olaniran et al., 2017). The highest concentration of these two alcohols was anyway in BH sample.

The concentration of acids was the lowest in the dry-hopping beers either in BF or in BH (Table 3), Caprilic acid was in the greatest amount among acids, especially where dry-hopping was not used, followed by hexanoic acid. These acids are known as long chain or medium chain fatty acids (LCFA or MCFA) and come from cell membrane thus indicate yeast degradation or are produced (i.e.octanoic and decenoic) in hypoxic conditions by the yeast *Saccharomyces cerevisiae* as by-products of its metabolism and are considered fermentation inhibitors in the presence of ethanol at acidic pH (Borrull et al., 2015). They are important in the formation of non-acetil esters playing an important role in the fruity aroma of beer. Add of dry-hopping in freeze-dried beer reduced significantly acids and consequently esters, reducing the aromatic potential.

Now, taking into account the data of alcohols, acids, and esters (as combination of the formers), the reduction in BF beer and especially with dry-hopping, could due to low yeast cell mass or less degradation of yeast cell during fermentation; indeed, it is wellknown during fermentation, especially initialy, the synthesis of fatty acids and to aminoacid or protein for the cell mass. Successively, the catabolism of nitrogen compounds, mainly aminoacids, produces higher alcohols, and a high measured content of MCFA indicate high membrane oxidation (Borrull et al., 2015; Liu et al., 2019). As aforementioned, a potential delay in the beginning of alcoholic fermentation could have created less yeast mass, and the highest antioxidant acitivity of freeze-dried hops could have protected the yeast cell during the increasing fermentation temperature, which is a very oxidative process (Bianchi et al., 2023b). The result is a less aroma-rich beer. This could explain also the less value of IBU because all the VOCs in solution in a closed tank for fermentation, with a low headspace, following the Henry law, tend to be diluted in the liquid. The gas–liquid partitioning of volatile compounds produced during fermentation is very important either for the yeast biochemistry or for the technological viewpoint (more aromatic matrix). Morakul et al. (2011) reported the partitioning is not influenced by the CO_2 production rate and is a function of only the must composition and the temperature.The partition coefficient (k) and phase ratio (β) affects the volatility of these hydrophobic compounds. Thus a higher production of VOCs in BH beer with a high partition coefficient due to the closed container could have accumulated VOCs affecting the bitterness of the beer. It is know that especially fatty acids can give a very unpleasant flavour in beer (Olšovská et al., 2019). We did not detect acetaldehyde which is the most important aldehyde in beer but it is an index of oxidation. We did not detect any diacetyl but we detected butanediol and acetoin which are the derived compounds, indicating a glyceropyruvic fermentation. As regards nonanal, it is a previous step of nonanoic acid (pelargonic acid) which found among cell membrane acids (Table 3). Styrene presence in hot stove samples is probably a contamination from the plastic tray used.

As regards, VOCs produced by secondary metabolisms, in the class of terpenes, 20 compounds were identified (Table 3). Beers produced with the dry-hopping technique, had a significantly higher content in terpenes, especially in beer from freeze-dried hops. 2,7-Dimethyl-2,6-octadiene was in significantly higher concentration than the other compounds usually characterizing hop (Mastrangelo et al., 2023; Steyer et al., 2017) but, the difference between dry-hopping and not, was marked by β-linalool and β-citronellol which were in double concentration in BFDH and BHDH samples. Freeze-drying and dry-hopping procedure provided the highest concentration of these aromatic compounds confirming the role of hops in add of terpenes, which are preserved by using the least invasive drying technique. Total furans were higher in BF samples while total volatile phenols,mainly 2-methoxy-4-vinylphenol and tyrosol in BH samples, regardless dry-hopping.

As shown in Figure 1, the first two principal components account for more than 90 % of the data variability (PC1 70.4 %, PC2 23.2 %), while the remaining three PCs explain the residual variance (PC3 6.4 %). Therefore, we have only reported the first 2 PCs. The PCA of VOCs clearly distinguished the beers depending on drying techniques (BH and BF). Dry-hopping (BFDH and BHDH) cancelled this difference. This observation could be attributed to the release of certain compounds (such as terpenes) or an increase in others do not present in beers without dry-hopping (BF and BH).

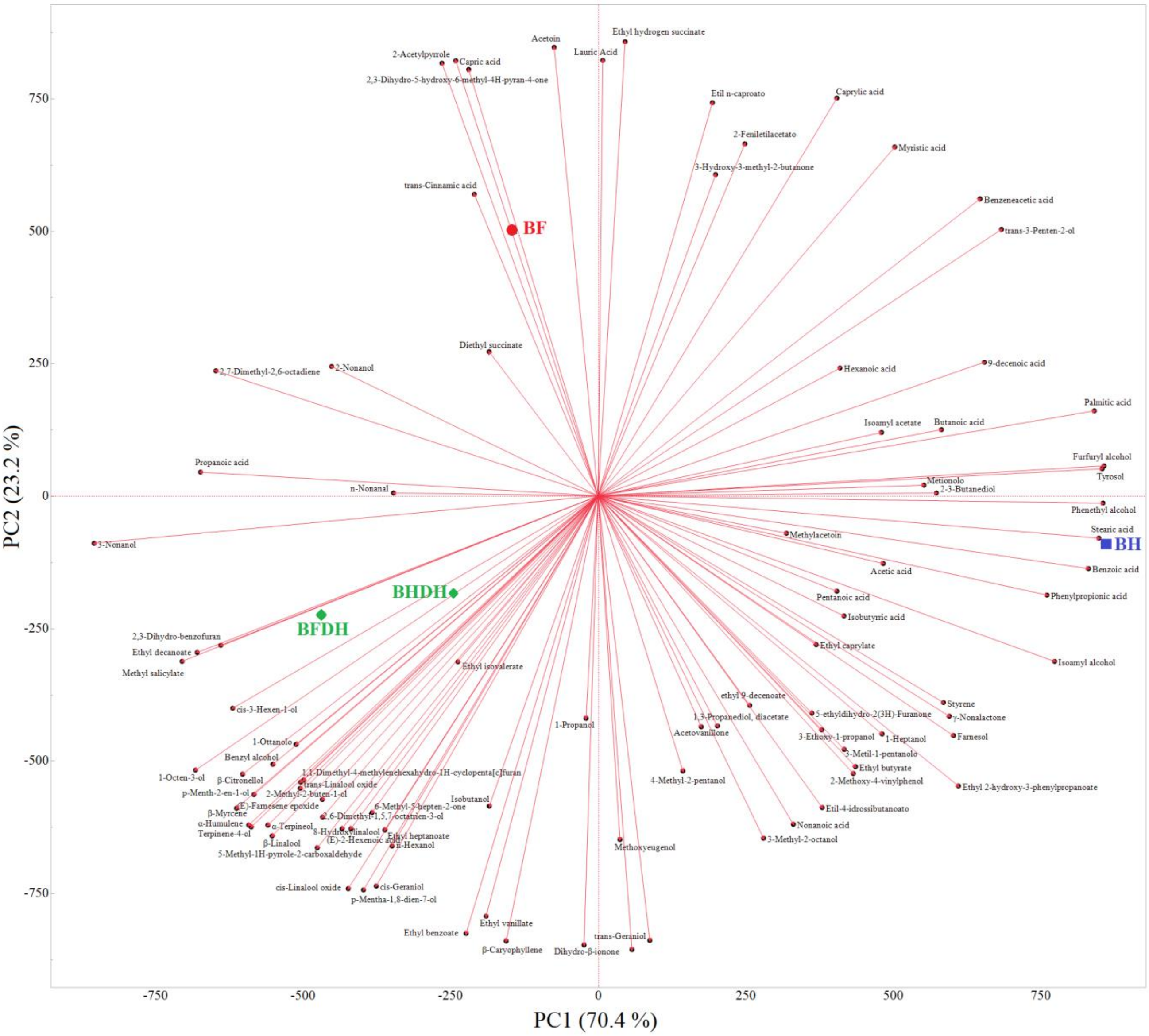

Figure 1. Biplot of Principal component analysis (PCA) of the volatile organic compounds (VOCs) of the beers. Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

The data of the PCA, are also confirmed by the hierarchical cluster analysis (HCA) reported in Figure 2. The HCA showed how the two beers with the dry-hopping (BHDH and BFDH) cluster together and have a profile separated from the other samples.
The beer without dry-hopping (BF and BH), are clearly separated in two different clusters, and this is correlated to the techniques of hop drying (F and H) which produce different aromatic compounds in the product, probably related to the drying temperature used.

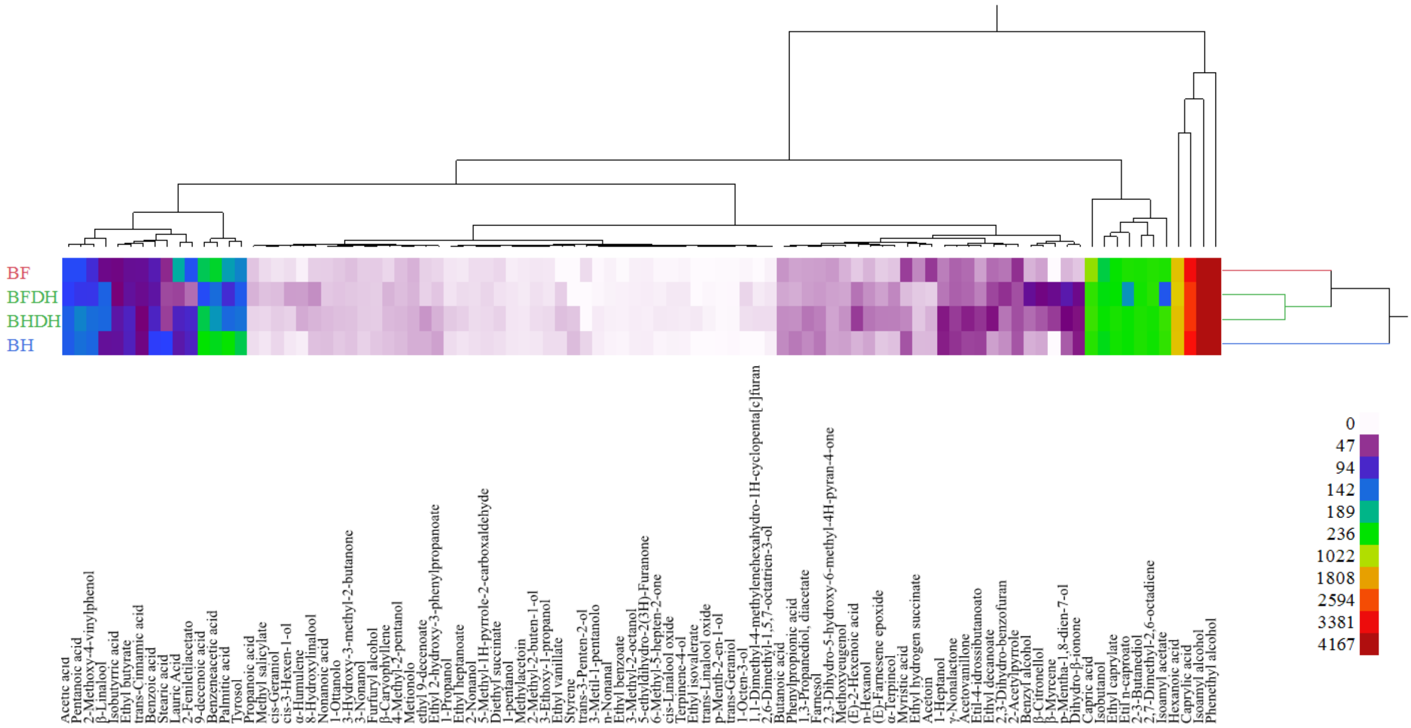

Figure 2. HCA of the volatile organic compounds (VOCs) of the beers. Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

3.3 Sensory evaluation

After sensory analysis, data were elaborated to define sensory profile of the samples and peculiarities due to the different types of hopping (Figure 3 a,b,c,d).

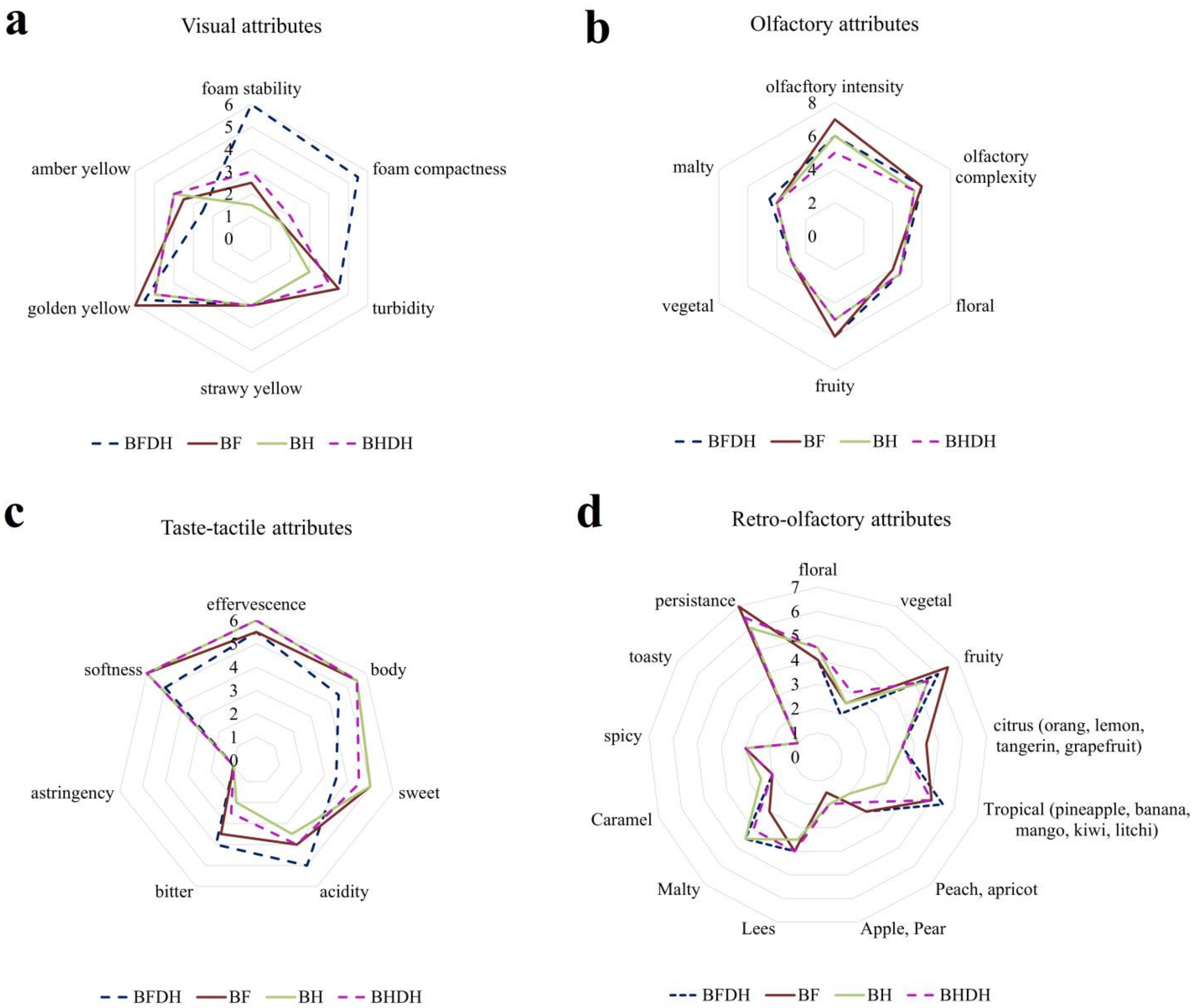

Figure 3. Sensory evaluation of beers: Visual attributes (**a**); Olfactory attributes (**b**);Taste-tactile attributes (**c**); Retro-olfactory attributes (**d**). Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

As general consideration, it can be stated that very low scores for vegetal, apple and pear, caramel and toasty notes are reported for all the analized samples; the same is for astringency in regard of the tactile perceptions as reported also in Medoro et al. (2016) for pilsner beer and from Carbone et al. (2021) working with the same variety of hops.

Considering BF sample, it was described as golden yellow with amber components and a presence of turbidity; foam was neither stable nor compact. The aroma profile at direct olfaction, was the most intense and complex of the set, characterized by a predominant fruity note. Beer was considered soft, medium effervescent, with a good sensation of body and sweetness; acidity and bitterness were low, as well as astringency. In the retro-olfactory perception, a persistent fruity and citrus notes prevailed upon the others.

Moving to the dry-hopped version, BFDH, the beer resulted more golden than with compact and stable foam and was as turbid a BF. The olfactory profile was less intense, maybe related with some problems with the aroma stability occurring when dry-hopping is applyde (Klimczak et al., 2023) but as complex as the previous one, characterized by floral, fruity and malty notes. Taste wise this BFDH was the most acidic and bitter sample confirming the expected inpact of higer concentrations of alpha-humulene in dry-hopped beers, and, beyondcomposition, also, because of lack of body, softness, and sweetness able to counterbalance these perceptions. The flavor profile was long as for BF and characterized by tropical fruits, malt, and lees scents.

The two beers produced with hot-stoved hop were the most ambered and the least golden in color, regardless the dry-hopping, with BH that was the poorest for foam stability and compactness, but also the least turbid. The amber color could be the effect of temperature favoring the formation of caramelized compounds, as well as the high content of VOCs included some phenols, thus not only an effect of oxidation but this could occure because the foam was not desirable, thus the protection of he CO_2 could be reduced. BHDH was comparable to BF in terms of foam and turbidity. In respect of the olfactory analysis, these two samples showed a lower complexity than the others and, particularly BHDH, also a lower intensity, with a bouquet characterized by more floral than fruity notes. Considering mouth perception, hot-stoved hop beers were the most effervescent and the softest with the least bitterness and, considering BH sample, the least acidity. Considering body, softness and sweetness these two samples were aligned with BF samples. The retro-olfactive profile was less complex than those for freeze-dried hop beer, more vegetal and lee notes as for BHDH and more malty and caramel like for BH. Apple and pear, even if at low level, were perceived as flavors of these beers. The tropical note is still present for the dry-hopper BHDH, confirming again the peculiarities driven by the hop and the hoppyng system, able to enrich the final product of a mixture of monoterpens and free fatty acids (Klimczak et al., 2023; Lafontaine & Shellhammer, 2018). Moving to the liking, the least liked beer was BHDH for all the different aspects; the other three samples were generally liked evenly, with BH picking for retro-olfactory liking and balance (Figure 4). The factors that led to the lower linking of BHDH sample could be the higher bittrenss, acidity and lowes sweetness found in the beer releted to less softness and a lower scructure in the body, resulting in a more watery and sharp products not supporting the retroolfactory perception. This is also confirmed by the fact that the most balanced and liked was BH, presenting high values of softness, sweetness and body, accompanied by medium bitterness and acidity.

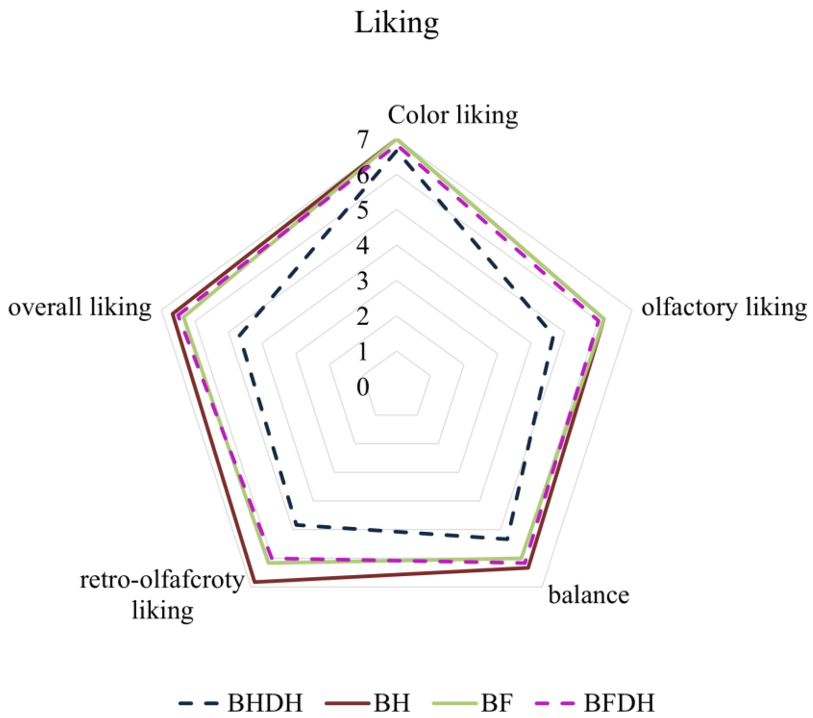

Figure 4. Sensory linking of beers. Beer with freeze-dried hop added during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

4. Conclusions

The research activity carried out showed a clear demarcation, both analytical and sensory, between the beers produced with different hop drying technique. Freeze-drying maintained a high content in hops polyphenols and antioxidant activity. Hops dried with hot stove produced beer with high ethanol and titratable acidity, and low in volatile acidity. Beers produced with the dry-hopping technique, had a significantly higher content in terpenes especially in beer from freeze-dried hops. BFDH was more golden than amber, with foam compactness and stability, and was as turbid a BF. The olfactory profile was less intense but characterized by floral, fruity and malty notes. Taste wise this BFDH was the most acidic and the most bitter sample. We emphasized that beers were different in chemical features, VOC content and VOC diversity, and in sensory analysis but none of them showed off-flavor and off-odor.

CRediT author statement

Edoardo Monacci: Formal analysis, Investigation. **Federico Baris:** Formal analysis, Investigation. **Alessandro Bianchi:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Fosca Vezzulli**: Formal analysis, Investigation, Writing - Original Draft. **Stefano Pettinelli:** Formal analysis, Investigation. **Milena Lambri:** Methodology,Validation, Data Curation, Writing - Review & Editing. **Fabio Mencarelli:** Conceptualization, Methodology, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization,. **Fabio Chinnici:** Validation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Chiara Sanmartin:** Conceptualization, Methodology, Validation, Writing - Review & Editing. Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration

Ethics statement: The research obtained the approval of the Ethics Committee of the University of Pisa (protocol n. 0088081/2023). The research was conducted according to the ethical guidelines and informed consent was obtained from all participants.

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

Data availability: Data will be made available on request.

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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:

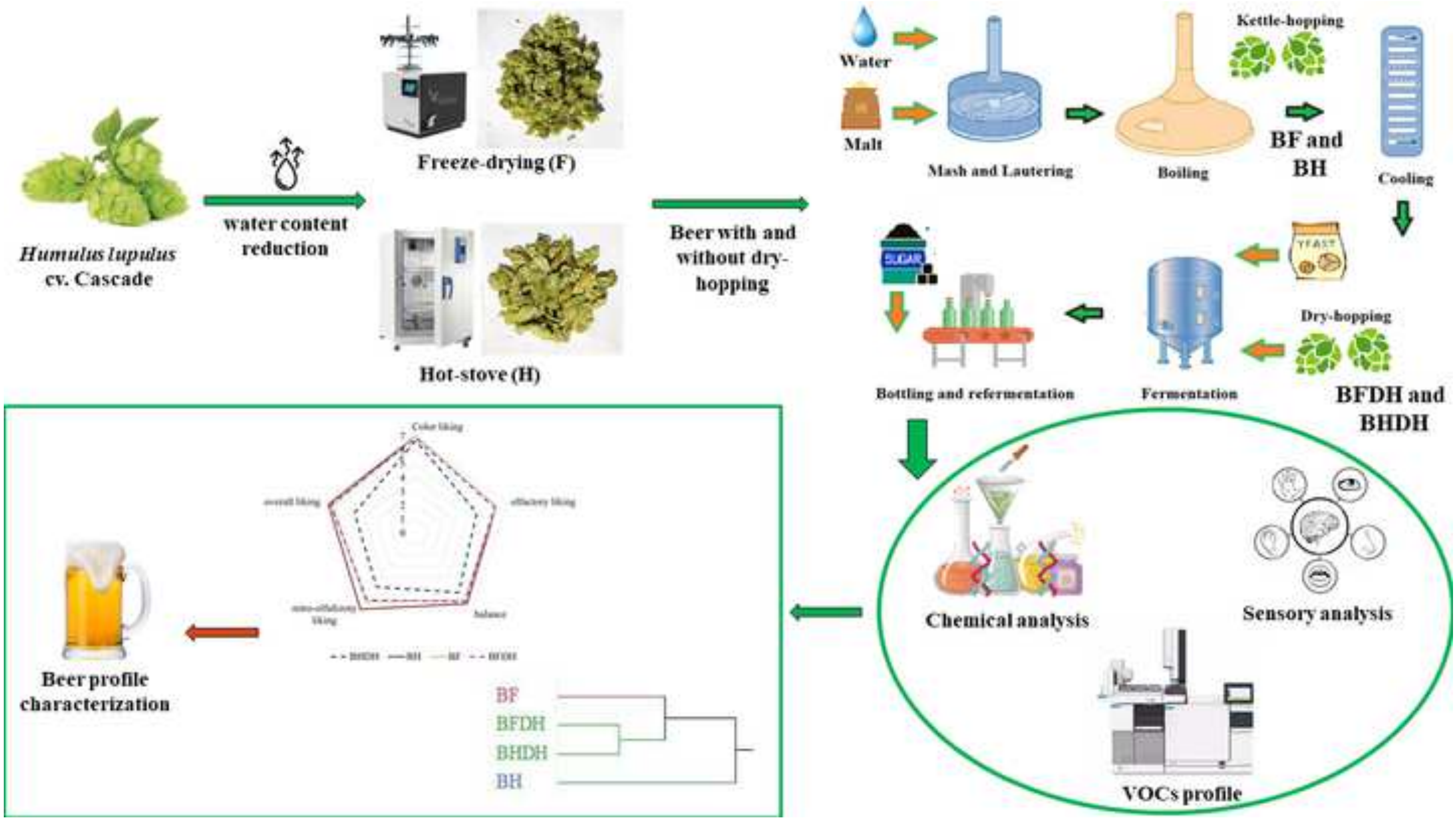

Humulus lupulus cv. Cascade
water content reduction
Freeze-drying (F)
Hot-stove (H)
Beer with and without dry-hopping
Water
Malt
Mash and Lautering
Boiling
Kettle-hopping
BF and BH
Cooling
YEAST
SUGAR
Dry-hopping
Fermentation
Bottling and refermentation
BFDH and BHDH
Chemical analysis
Sensory analysis
VOCs profile
Color liking
olfactory liking
balance
overall liking
BHDH
BH
BF
BFDH
Beer profile characterization

Figure 1

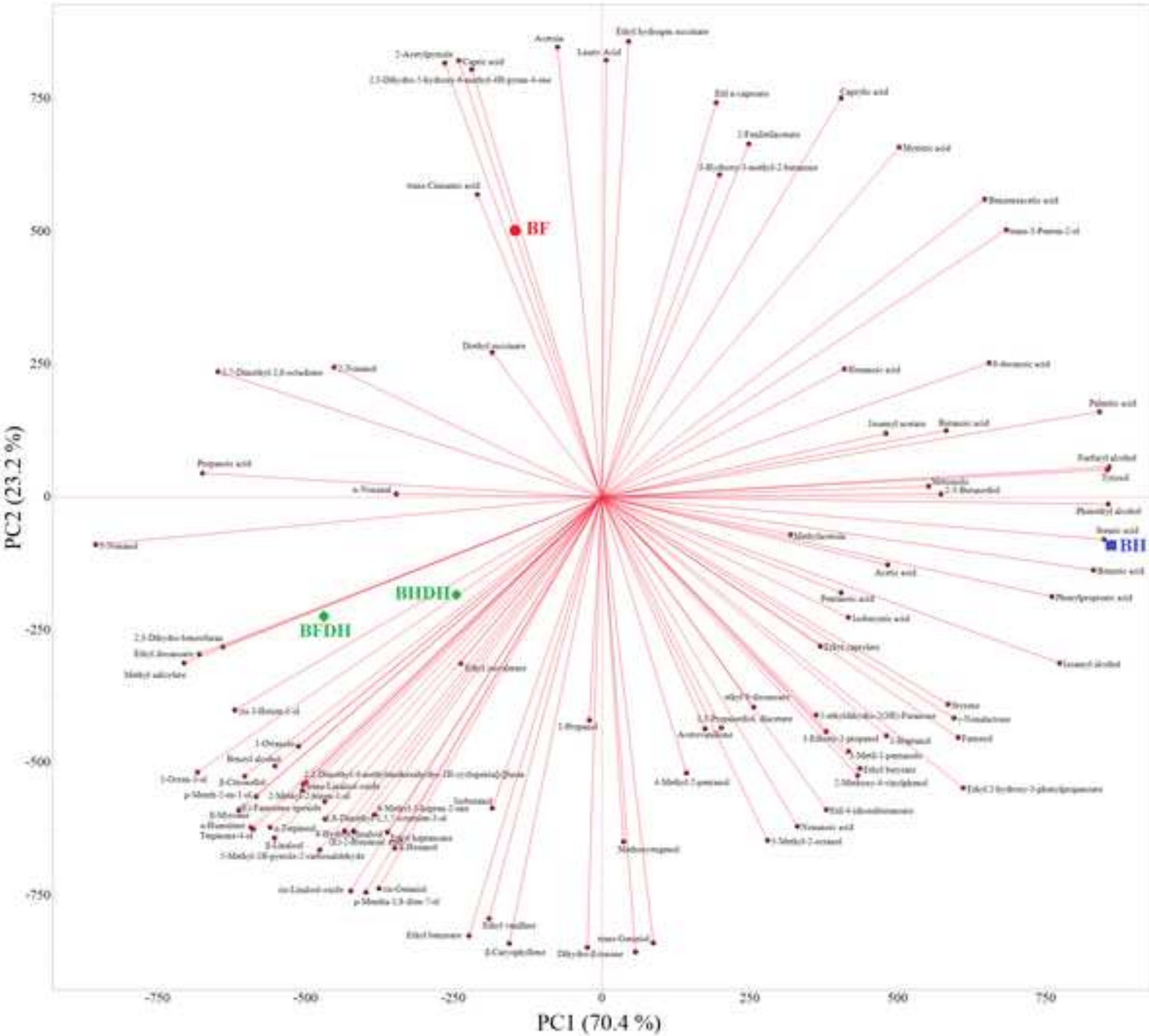

BF
BH
BHDH
BFDH
750
500
250
0
-250
-500
-750
PC2 (23.2 %)
-750
-500
-250
0
250
500
750
PC1 (70.4 %)

Figure 2

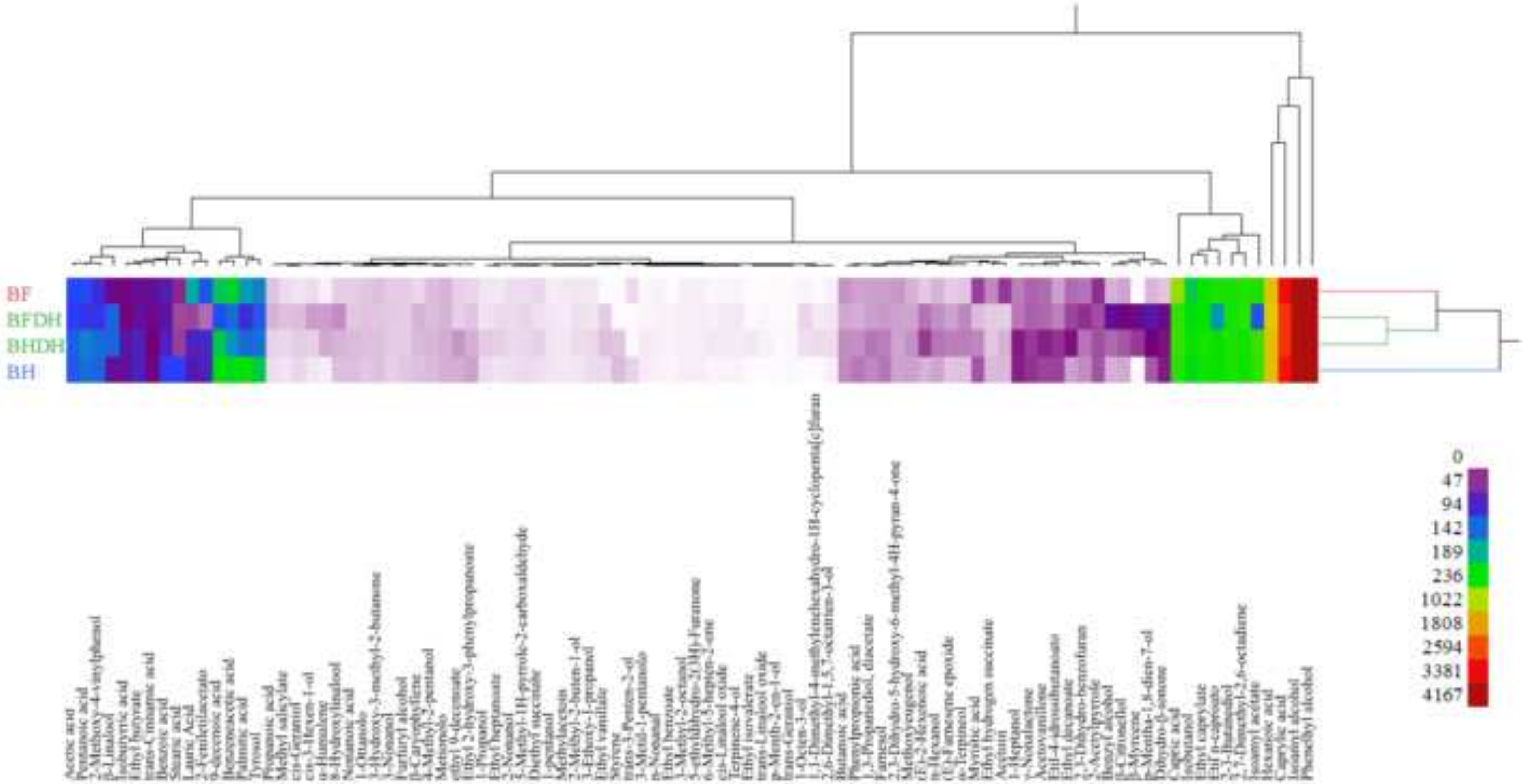


Figure 3

Click here to access/download;Figure(s);Figure 3a,b,c,d.tif

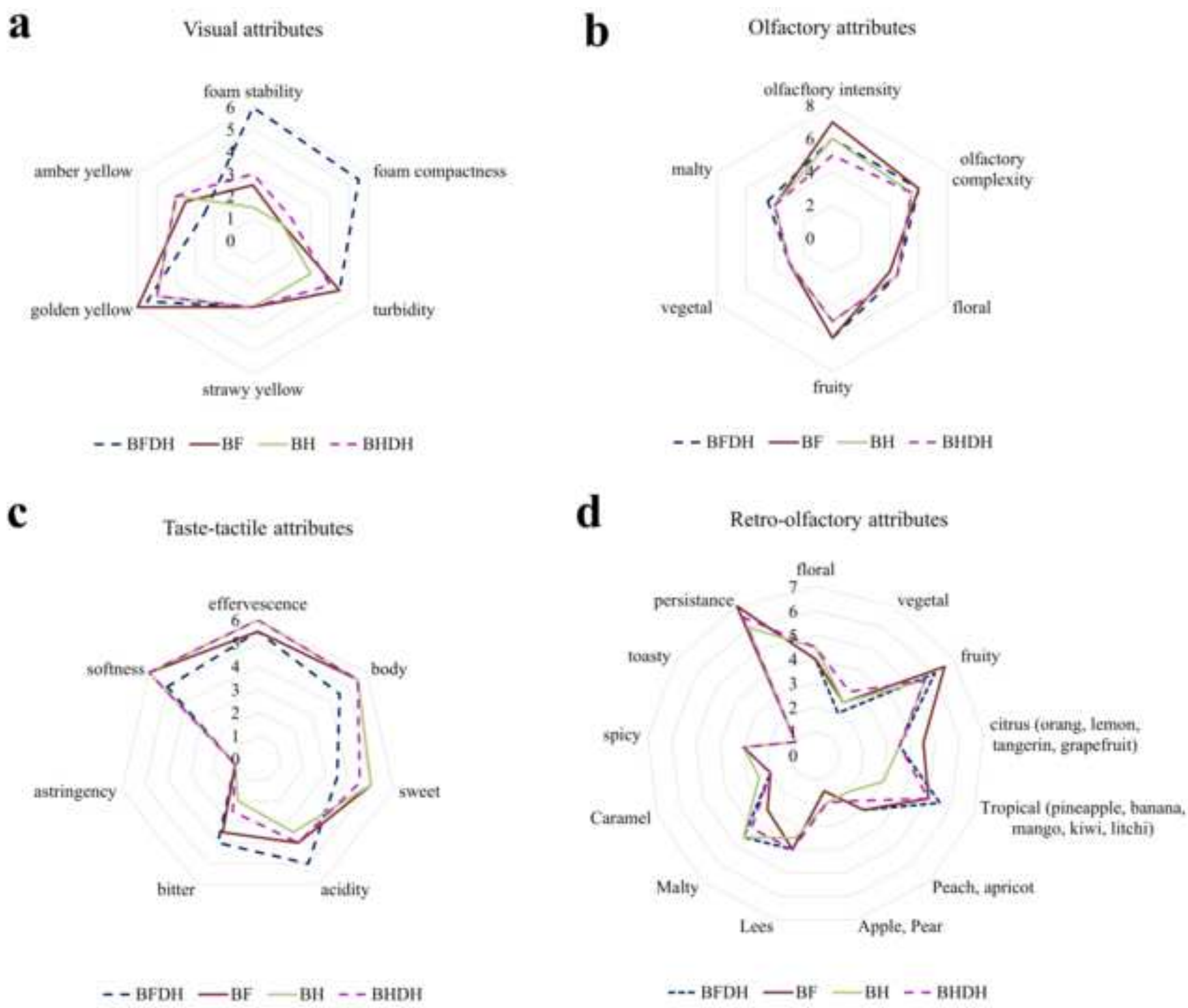
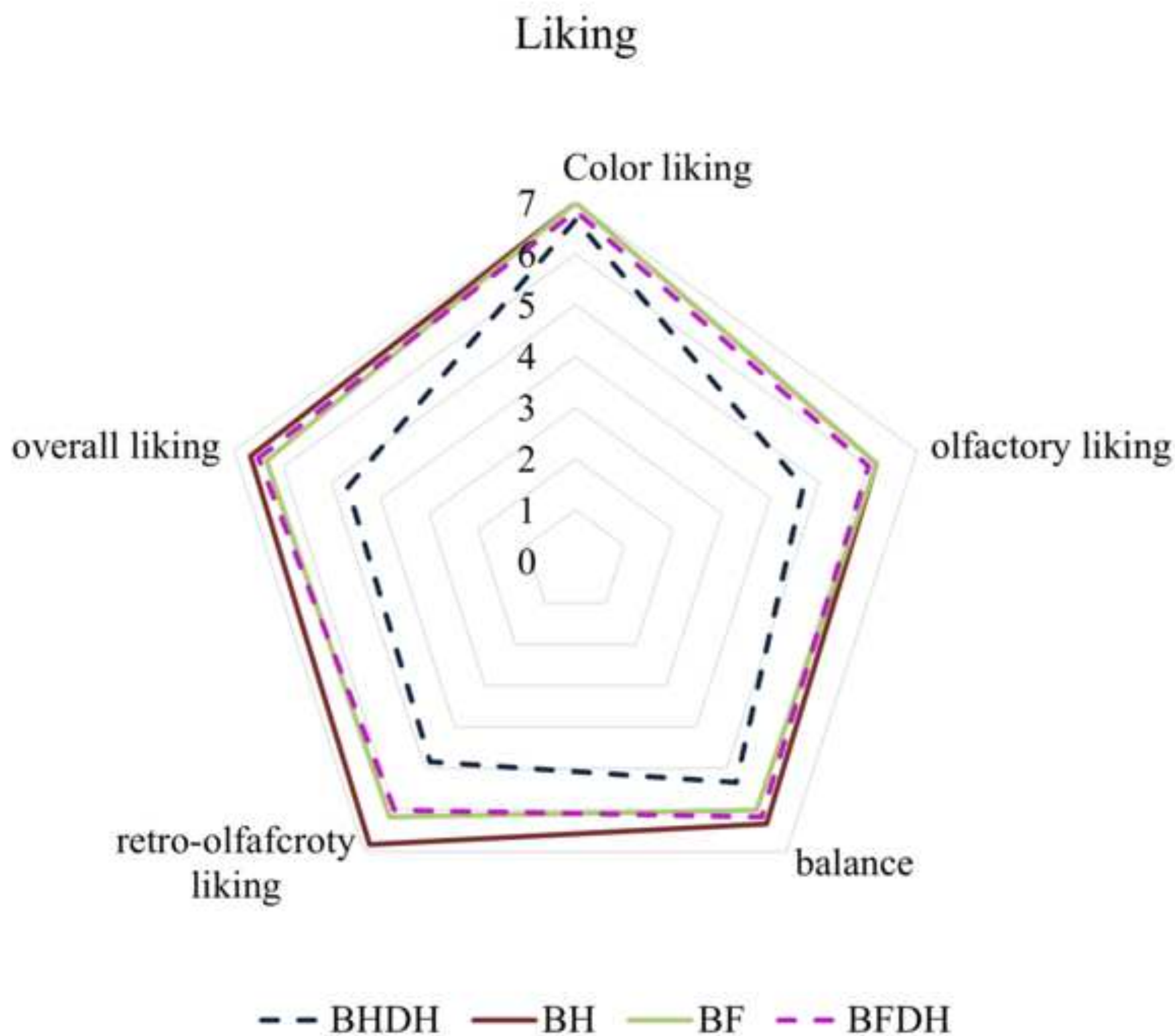


Figure 4


Liking
Color liking
olfactory liking
balance
retro-olfafcroty liking
overall liking
7
6
5
4
3
2
1
0
BHDH
BH
BF
BFDH