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

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Abstract:	Drying techniques are important to hop storage, but significantly affect the quality. Another important factor is the stage of hop addition in beer. Dry-hopping, adding hops during fermentation or conditioning, is a valid technique to enhance beer flavor. This study focuses on the impact of two drying techniques [freeze-dryer (F) and hot-stove (H)] for Cascade hop on the chemical, aromatic and sensory quality of beer, comparing beers produced without (BF and BH) and with dry-hopping (BFDH and BHDH). The dry-hopping significantly increased the bitterness index and reduced the titratable acidity. Isoamyl acetate and ethyl caprylate was high especially in BH while ethyl-n-caproate was the highest in BF. Beers with the dry-hopping had a significantly higher content in terpenes especially in BFDH. Sensory evaluation indicates varied preferences, with freeze-dried hop beers generally favored. Finally, depending on the type of beer, the different dried hops and the hopping technique can be chosen.
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Editor, *Food Chemistry*

4th March 2024
Pisa, Italy

Submission of research article for publication in *Food Chemistry*

Dear Editor,

We hereby submit our research article “**Influence of the drying process of Cascade hop and the dry-hopping technique on the chemical, aromatic and sensory quality of the beer**” by Monacci et. al., to be considered for publication.

Abstract:

Drying techniques are important to hop storage, but significantly affect the quality. Another important factor is the stage of hop addition in beer. Dry-hopping, adding hops during fermentation or conditioning, is a valid technique to enhance beer flavor. This study focuses on the impact of two drying techniques [freeze-dryer (F) and hot-stove (H)] for Cascade hop on the chemical, aromatic and sensory quality of beer, comparing beers produced without (BF and BH) and with dry-hopping (BFDH and BHDH). The dry-hopping significantly increased the bitterness index and reduced the titratable acidity. Isoamyl acetate and ethyl caprylate was high especially in BH while ethyl-n-caproate was the highest in BF. Beers with the dry-hopping had a significantly higher content in terpenes especially in BFDH. Sensory evaluation indicates varied preferences, with freeze-dried hop beers generally favored. Finally, depending on the type of beer, the different dried hops and the hopping technique can be chosen.

On behalf of my co-authors, I declare that this paper has not been published and is not being considered for publication elsewhere, and that, if accepted, the manuscript will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher.

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Yours sincerely,

Dr. Alessandro Bianchi



Highlight:

- Influence of hop drying technique and dry-hopping on beer quality.
- The drying technique affect beer aromatic profile.
- Dry-hopping influences acidity, polyphenols and antioxidant content of beer.
- Dry-hopping increases the beer aromatic characteristics.
- Depending on the beer type, the dried hop and hopping technique can be chosen.

1 *Research papers*

2 **Influence of the drying process of Cascade hop and the dry-hopping technique on the chemical, aro-**
3 **matic and sensory quality of the beer**

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16 of hop addition in beer. Dry-hopping, adding hops during fermentation or conditioning, is a valid technique to enhance beer flavor.
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18 aromatic and sensory quality of beer, comparing beers produced without (BF and BH) and with dry-hopping (BFDH and BHDH).
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20 was high especially in BH while ethyl-n-caproate was the highest in BF. Beers with the dry-hopping had a significantly higher
21 content in terpenes especially in BFDH. Sensory evaluation indicates varied preferences, with freeze-dried hop beers generally
22 favored. Finally, depending on the type of beer, the different dried hops and the hopping technique can be chosen.
23

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

25 **1. Introduction**

26 Hop (*Humulus lupulus* L.) is an essential raw material for beer production as it provides an increased shelf-life, bitterness, and aroma
27 to beer (Heřmánek et al., 2018; Raut et al., 2021; Steinhaus & Schieberle, 2000). The quality of the raw material is strongly influ-
28 enced by the post-harvest transformation methodologies. In fact, the hops, following harvesting, are unsuitable for use as they are
29 in the brewing process. In order to be marketed, as well as used in the beer production process, the raw material must undergo
30 particular conditioning and transformation methods. The water concentration inside the fresh inflorescences (moisture) is about 80
31 % (Monacci et al., 2023; Verzele & De Keukeleire, 1991) and in order to be marketed and used in the beer production process, the
32 raw material must undergo a drying process (Neve, 2012; Rybacek, 2012) to reduce the water content down to 8 - 11 % (Heřmánek
33 et al., 2018; Rybka et al., 2018).

34 In this regard, it is important that the matrix undergoes a drying process in the shortest time, in order to preserve its quality and
35 increase its shelf-life (Rossini et al., 2021). The temperature at which the drying process is conducted inevitably determines the loss
36 of chemical compounds present within the inflorescence (Rybka et al., 2018). The quantity of essential oils presents in the fresh
37 product can be reduced by 30-40% following processing, due to the volatility of these compounds (Nance & Setzer, 2011; Rybka et

38 al., 2018). For this reason, the control of the temperature at which the process is carried out is of extreme importance with regard to
39 the quality of the final product for beer production (Raut et al., 2020).

40 Another important factor in beer process, is the stage of hop addition to beer. There is no doubt on the significant contribution of
41 dry-hopping to the renaissance of craft beer and the subsequent boom in sales of craft beers around the world (Maye et al., 2018;
42 Oladokun et al., 2017). Dry-hopping represents a relatively simple means of improving the beer flavor; brewers add between 2 – 12
43 g/L of hops in the form of cones or pellets into beer during fermentation or conditioning for periods ranging from several days to
44 weeks (Hauser et al., 2019; Lafontaine & Shellhammer, 2019). The added hops can be left in the beer with no mixing (static dry-
45 hopping) or with mixing, i.e. using a pump or CO₂ (dynamic dry-hopping). Perhaps one of the unintended consequences of dry-
46 hopping is the effect this process has on perceived beer bitterness. Several studies have shown an increase in both measured analyt-
47 ical and perceived bitterness in dry-hopped beers (Algazzali & Shellhammer, 2016; Lafontaine & Shellhammer, 2019; Maye et al.,
48 2016). Brewers can also add hop oil essences to beer to create specific flavor characters that mimic dry-hopped flavor in their
49 product (Gomes et al., 2022; Klimczak et al., 2023). The increase in aroma perceived in dry-hopped beers versus conventionally
50 hopped beers is thought to be due to elevated levels of several volatile terpene compounds, hydrocarbons and their derivatives e.g.
51 linalool, myrcene, humulene, β -citronellol and geraniol (Klimczak et al., 2023; Oladokun et al., 2017; Takoi et al., 2010). Since
52 these chemicals are rapidly lost through evaporation during wort boiling or fermentation, they are seldom perceived in beers that
53 are not dry-hopped (Lafontaine & Shellhammer, 2019). Furthermore, the presence of yeast during dry-hopping adds an extra level
54 of complexity to this process. Some researchers have reported the biotransformation of certain volatile hop compounds during and
55 post fermentation (maturation), e.g., in the conversion of geraniol into β -citronellol (Takoi et al., 2010). This means that brewers
56 must also decide whether to totally remove yeasts from beer before dry-hopping. The presence of yeast during dry-hopping may
57 offer other benefits: suspended yeast can metabolize dissolved oxygen during dry-hopping, thereby protecting beer and hops vola-
58 tiles from oxidation during the process (Gomes et al., 2022; Oladokun et al., 2017).

59 Thus, our hypothesis when we started the research project was that the effect of water removal (drying with freeze-dryer or hot-
60 stove) could affect the quality of beer especially in term of VOCs (volatile organic compounds) when used dry-hopping.

61 2. Materials and Methods

62 2.1 Raw material

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

69 For each water reduction technique, approx. 10 kg of fresh hop inflorescences were used, divided in 3 trays to place in the stove or
70 in freeze-dryer. Chemical parameters of hop were reported based on dry matter (dm) of sample (Table 1), determined in triplicate
71 on approximately 10 g (from each tray), drying at 105 °C until constant weight (Bianchi et al., 2024).

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

Chemical parameters	Units	F	H
α -acid	(%)	7.17 \pm 0.09	7.29 \pm 0.08
β -acid	(%)	5.72 \pm 0.05	6.73 \pm 0.03
HSI	-	0.18 \pm 0.02	0.23 \pm 0.01
Total polyphenols	(g GAE/kg dm)	442.61 \pm 3.31	339.48 \pm 1.03
ABTS	(mmol TE /kg dm)	34.42 \pm 0.49	22.32 \pm 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

74

75 **2.2 Beer production**

76 The ingredients used for beer production (each beer-must) were: 10 kg of malt Maris Otter (Muntons, Stowmarket, United King-
77 dom), 10 kg of malt Pilsner (Weyermann, Bamberg, Germany), 300 g of dried hop (F and H), 15 g of dry yeast SafAle™ US-05
78 (Fermentis, Marquette-lez-Lille, France) and water (70 L in mash, 63.6 L in sparge). Beer was produced in spring 2023 in a stainless-
79 steel plant Easy 100 (Polsinelli Enologia Srl, Isola del Liri, Italy) to produce 100 L of beer, following the phases described in
80 Mastrangelo et al. (2023): (i) mash: insertion of ground malts at 35 ± 2 °C; (ii) protein rest: 55 °C for 10 minutes; (iii) mash-in: 65
81 °C for 50 minutes; (iv) β–glucan rest: 72 °C for 10 minutes; (v) mash-out: 78 °C for 5 minutes; (vi) Sparge with water at 78 °C for
82 the sugar extraction from brewers grains; (vii) the must was divided into 2 tanks and boiled for 60 minutes at 95 ± 2 °C; (viii) during
83 the boiling was added hops in 3 portions (100 g) every 20 minutes; (ix) the must was cooled (23 ± 2 °C) and the yeast was inoculated
84 (15 g/hL).

85 The dry products (300 g each) from 2 different techniques (freeze-dryer (F), hot-stove (H)) was used as aforementioned.

86 For the fermentation phase, the beer-must was divided into 4 (two each sample) fermenters of 20 L:

- 87 1- Beer with freeze-dried hop added during the boiling phase (BF).
- 88 2- Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH).
- 89 3- Beer with hot-stove dried hop added during the boiling phase (BH).
- 90 4- Beer with hot-stove dried hop added during the boiling phase and dry-hopping (BHDH).

91 The primary fermentation lasted 16 days, 11 days at 18 ± 2°C and 5 days at 6 °C ± 1°C in cold room to allow the precipitation of
92 the solid compounds (lagering). On the 7th day of fermentation, a static dry-hopping for 48 hours (concentration of 5 g/L) has been
93 done in the BFDH and BHDH beer-must. Successively, the beer was bottled in 0.5 L glass bottles and capped with a crown cap.
94 During the bottling phase, 6 g/L of commercial beet sugar were added to start the bottle secondary fermentation which lasted one
95 month at 8 °C ± 1°C.

96 **2.3 Chemical characterization of hop**

97 The chlorophyll and carotenoid contents [g/kg on dry matter (dm)] were determined according to Monacci et al. (2023).

98 The total polyphenols (g of gallic acid equivalents (GAE)/kg dm) and the anti-radical activity (ABTS and DPPH free radical method)
99 (mmol Trolox equivalents (TE)/kg dm) were determined as previously reported (Bianchi et al., 2023a).

100 The concentration of α- and β-acids (%) and the Hop Storage Index (HSI) was calculated according to previously reported (Monacci
101 et al., 2023).

102 The quantification of the essential oil yield of the samples was carried out in agreement with Van Simaey et al. (2022) utilized the
103 hydro-distillation process and calculated as reported in the following equation:

$$104 \text{ Yield essential oils (\% V/w)} = \frac{\text{Volume hydrosol (mL)}}{\text{sample weight (g)}} \times 100$$

105 **2.4 Chemical analysis of beer**

106 Beer chemical analyses were carried out on four, 0.5 L bottles, two from each fermenter vat (4 bottles each beer production), and
107 before analyses, the beers were degassed by ultrasound. Chemical parameters: alcohol (% V/V), pH, residual sugars (g/L hexoses),
108 titratable acidity (g/L lactic acid), volatile acidity (g/L lactic acid), total polyphenols (mg/L gallic acid), color according to the SRM

109 (Standard Reference Method) scale and bitterness according to the IBU (International Bitterness Unit) scale, were carried out fol-
110 lowing the official method of the American Society of Brewing Chemists as previously reported (Mastrangelo et al., 2023). Finally,
111 the anti-radical activity of beer (mmol Trolox equivalents (TE)/L) was determined by DPPH and ABTS free radical methods as
112 reported (Bianchi et al., 2023c).

113 **2.5 Volatile organic compounds (VOCs)**

114 The analysis of VOCs of beer was performed as previously reported (Mastrangelo et al., 2023). In particular, 10 mL sample of beer
115 (2 bottles each beer production), degassed by ultrasound, and 100 μ L of a 2-octanol solution at 500 mg/L was added as an internal
116 standard. The sample was deposited on a Hypersep Retain Prep (Thermo Fisher Scientific, Milan, Italy) cartridge (60 mg), activated
117 with 2 mL dichloromethane, 2 mL methanol and 2 mL water. The analytes were eluted with 5mL of dichloromethane, collected in
118 soviel on the bottom of which 2 grams of anhydrous sodium sulfate had been inserted and placed in the freezer overnight. Samples
119 were filtered with a cellulose filter to remove sodium sulfate and concentrated to a final volume of 200 μ L under a stream of N₂.
120 The GC apparatus consists of a Trace GC ultra-gas chromatograph with a Trace DSQ with quadrupole mass detector (Thermo Fisher
121 Scientific, Milan, Italy) and a Stabilwax DA capillary column (Restek, Bellefonte, PA, USA; 30 m, 0.25mm i.d., and 0.25 μ m film
122 thickness) and He (constant flow of 1.0 mL/min) was used as The carrier gas.

123 GC temperature ramp was programmed as reported by Castro-Marín et al. (2018): from 45°C (maintained for 1 minute) to 100°C
124 (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
125 in splitless mode and the volume injected is 1 μ L. Analyses were done in quadruplicate (four bottles) and GC-MS parameters were
126 obtained by using Xcalibur v 4.1 software (Thermo Fisher Scientific, Milan, Italy). Identification and the quantification of com-
127 pounds (μ g/L) were carried out as previously reported (Mastrangelo et al., 2023).

128 **2.6 Sensory analysis**

129 The beer sensory profile was evaluated by a panel of ten beer experts using QDA (qualitative discriminant analysis) method (Stone
130 et al., 2004) at the SensoryLab of DiSTAS of Università Cattolica del Sacro Cuore compliant with the International Organization
131 for Standardization standard (ISO 8589:2007) (Donadini et al., 2013). The samples were served randomly during the test with a
132 rotated plan. The sensory scoresheet presented five main classes of parameters selected and slightly modified from Donaldson et al.
133 (2012) and Donadini et al. (2013): visual, olfactory, tactile, gustatory and retro-olfactory perception. The first class was divided
134 into: foam stability, foam compactness, turbidity, yellow straw color, golden yellow color, amber yellow color, and color pleasant-
135 ness. The second class was divided into: olfactory intensity, olfactory complexity, floral, fruity, vegetable, malty and olfactory
136 pleasantness. The third class was divided into: effervescence, body, sweet, sour, bitter, astringency, softness and balance. The last
137 class was divided into flowers, vegetables, fruits, citrus fruits (orange, lemon, tangerin and grapefruit), tropical fruits (pineapple,
138 banana, mango, kiwi and lychee), peach/apricot, apple/pear, yeast, malt, caramel, spicy, toasted, persistence, DMS-defects, mold-
139 defects, cardboard-defects and retro-olfactory pleasantness. For all the samples, after the objective evaluation, the overall liking of
140 the sample was evaluated. The intensity of each attribute was evaluated on a nine-point horizontally oriented scale anchored as ‘‘not
141 perceived at all’’ and ‘‘extremely intense’’. Before starting the analysis, panel was calibrated around the median \pm 1, calculated for
142 each attribute, after the evaluation of an extra sample served as calibration sample. Repeatability, discrimination and collimation of
143 the panel and panelists were evaluated thanks to the presence of an analytical replicate in the set (Vezzulli et al., 2021).

144 **2.7 Statistical analysis**

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

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

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

151 3. Results and Discussion

152 3.1 Chemical characterization of beers

153 BH and BHDH samples have a significantly higher alcohol content than BF and BFDH samples (8.85% v/v for BH and 8.42% v/v
 154 for BHDH vs 6.34% v/v and 6.24% v/v in BF and BFDH) (Table 2) while no significant difference was found in residual sugar
 155 concentrations. Titratable acidity was higher in samples BH and BHDH and dry-hopping reduced the acidity. The observed differ-
 156 ence between F and H samples could be due to the degree of sterilization of the matrix. The freeze-drying technique guarantees a
 157 lower depletion of the bioactive components of biological matrices, with an increase in product quality (Karam et al., 2016) but, in
 158 contrast, freeze-drying does not guarantee the complete removal of microorganisms (Yadav and Roopesh, 2020). Gram-positive
 159 bacteria have a greater ability to survive than gram-negative ones in freeze-drying (Morgan and Vesey, 2009; Wang et al., 2023).
 160 Thus the higher ethanol content of BH and BHDH could be due to by microbial competition during fermentation, consuming the
 161 same amount of sugars but affecting different metabolic processes (Romero-Rodríguez et al., 2022). Also the reduction of titratable
 162 of titratable acidity in BF and even more in BFDH can be related to the presence on hop inflorescences of bacteria, which have
 163 consumed the acids present in solution. Moreover, it is well documented in the literature that there is an increase in pH with dry-
 164 hopping, and a consequent lowering of total acidity (Hauser et al., 2019; Lafontaine & Shellhammer, 2018; Rutnik et al., 2022).
 165 The correlation can be observed if we take into consideration the concentration of acetic acid and the alcohol content in the product
 166 (Table 2). In this case, in the BF and BFDH samples there is an increase in volatile acidity, which is 0.23 g/L and 0.41 g/L respec-
 167 tively, and a reduction in ethanol concentration. The appearance could be determined by a microbial component on the lyophilized
 168 product, as aforementioned, that favors the formation of acetic acid from the substrates present in solution. In BH and BHDH
 169 samples the presence of acetic acid is much smaller, presenting values of 0.17 g/L and 0.14 g/L respectively.

170 The color of the beer is determined by the interaction between the different raw materials of the recipe, as well as by the temperatures
 171 at which the mashing phase is conducted, and by the possible enzymatic oxidation of polyphenols (Koren et al., 2020; Pahl et al.,
 172 2016; Pettinelli et al., 2022). The SRM (Table 2) values fell into the Amber category, which has an SRM of 13-16. Beers produced
 173 through the use of hops dried with a hot stove at a temperature of 40 °C (BH: 13.92; BHDH: 13.32), were slightly lighter than those
 174 with freeze-dried hops (BF 16.09; BFDH 16.15). The cause is attributable to a more marked oxidation of polyphenols in BF and
 175 BFDH samples, whose starting material had a higher content in this class of compounds (Table 1). In fact, the color of beer is
 176 conditioned by the oxidation of monomers of the flavonols class and the oligomers of proanthocyanidins (Koren et al., 2020).

177 **Table 2.** Main chemical parameters of beers. Data are the mean (\pm SD) of 4 bottles. Beer with freeze-dried hop added during the
 178 boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-stove dried
 179 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 \pm 0.11 b	6.26 \pm 0.14 b	8.85 \pm 0.17 a	8.42 \pm 0.12 a
pH	-	4.68 \pm 0.02 b	4.84 \pm 0.03 a	4.50 \pm 0.03 c	4.63 \pm 0.04 b
Residual sugars	g/L hexoses	0.47 \pm 0.07 a	0.35 \pm 0.06 a	0.39 \pm 0.02 a	0.42 \pm 0.03 a
Titratable acidity	g/L lactic acid	1.66 \pm 0.15 b	1.23 \pm 0.12 c	1.97 \pm 0.13 a	1.77 \pm 0.19 ab
Volatile acidity	g/L acetic acid	0.23 \pm 0.02 b	0.41 \pm 0.05 a	0.17 \pm 0.04 bc	0.14 \pm 0.03 c
Color (SRM)	-	16.09 \pm 0.52 a	16.15 \pm 0.62 a	13.92 \pm 0.41 b	13.32 \pm 0.42 b
IBU	-	10.43 \pm 0.63 b	6.26 \pm 0.51 d	8.93 \pm 0.27 c	16.46 \pm 0.58 a
Total polyphenols	mg/L gallic acid	395 \pm 25 ab	421 \pm 31 a	315 \pm 9 c	352 \pm 21 b
ABTS	mmol TE/L	1.34 \pm 0.02 b	1.60 \pm 0.05 a	1.26 \pm 0.03 b	1.34 \pm 0.04 b
DPPH	mmol TE/L	0.82 \pm 0.03 b	0.96 \pm 0.02 a	0.76 \pm 0.04 b	0.81 \pm 0.03 b

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

181 In BHDH (16.46) the dry-hopping technique significantly increased the bitterness index compared to BH (8.93). The IBU is deter-
 182 mined by the concentration of iso- α -acids present in solution, which determine the characteristic bittering power of the product
 183 (Bocquet et al., 2018; Pahl et al., 2016; Rutnik et al., 2022). The use of this hop infusion technique tended to increase the IBU value
 184 of the final product. The appearance is related to the oxidation of α -acids with the production of humulinone, which entering into
 185 solution contributes to the increase of the IBU (Hauser et al., 2019; Salamon et al., 2022). The beers produced with freeze-dried
 186 hops had an opposite trend compared to the aforementioned ones.
 187 In fact, the value in IBU decreased as a result of the dry-hopping technique, going from 10.43 in BF to 6.26 in BFDH. The loss in
 188 iso- α -acids is influenced by several factors, such as the absorption of these compounds by lees materials or yeast cells and their
 189 transformation which causes oxidative processes (Van Cleemput et al., 2009). Moreover, through dry-hopping a reduction of iso- α -
 190 acids is observed because they are replaced by oxidation products with less bittering power, thus IBU reduction (Maye et al., 2018).
 191 Finally, the results of total polyphenols and antioxidant power (ABTS and DDPH) show that BF and BFDH samples had higher
 192 values than BH and BHDH (Table 3). The results are affected by the quality of the starting matrix (Table 1), which had different
 193 values in total polyphenols, ABTS and DPPH, higher in BF and BFDH. The quality of the freeze-dried matrix is also influencing
 194 the antioxidant power of the finished product, especially following the dry-hopping technique (BFDH 1.60 mmol TE/L), while
 195 among the other samples, no significant differences (BH 1.26 mmol TE/L, BHDH 1.34 mmol TE/L, BF 1.34 mmol TE/L) were
 196 measured. In any case, the addition of hops following fermentation influences the amount of total polyphenols and antioxidant
 197 power. This aspect determines a greater stability of the chemical-physical characteristics of the finished product over time, increas-
 198 ing its shelf-life (C. W. Bamforth, 2016). Moreover, through the techniques of late hopping and dry-hopping, the extraction of α -
 199 acids in solution is favored (Oladokun et al., 2017) with the result of greater stability against microbiological contamination (Michel
 200 et al., 2020).

201 3.2 VOCs profile in beers

202 As in all fermented beverages, apart from ethanol and carbon dioxide which are the main products of fermentation, there are also
 203 classes of VOCs in beer which are formed as secondary products of fermentation and characterize its quality. Olaniran et al. (2017)
 204 reported what they define as “Flavour-active volatile compounds in beer”. Higher alcohols and esters represent the most important
 205 classes being the result of fermentation by yeasts. Among the esters, 1/3 is represented by ethyl acetate (Jespersen & Jakobsen,
 206 1996) which if in low concentration provides solvent nuance. Other classes in lower concentrations but important from an aromatic
 207 point of view are terpenes, furans, ketones.

208 **Table 3.** Volatile Organic Compounds (VOCs) in beers. Data are the mean (\pm SD) of 4 bottles. Beer with freeze-dried hop added
 209 during the boiling phase (BF); Beer with freeze-dried hop added during the boiling phase and dry-hopping (BFDH); Beer with hot-
 210 stove dried hop added during the boiling phase (BH); Beer with hot-stove dried hop added during the boiling phase and dry-hopping
 211 (BHDH).

VOCs ($\mu\text{g/L}$)	BF	BFDH	BH	BHDH
Ethyl butyrate	71.75 \pm 2.67 b	75.05 \pm 2.11 b	88.85 \pm 3.19 a	90.57 \pm 5.87 a
Ethyl isovalerate	n.d.	n.d.	n.d.	4.18 \pm 0.23 a
Isoamyl acetate	361.63 \pm 21.24 b	130.87 \pm 11.46 c	450.60 \pm 14.03 a	478.83 \pm 21.52 a
Ethyl <i>n</i> -caproate	359.37 \pm 9.73 a	164.22 \pm 6.91 c	263.43 \pm 13.56 b	265.91 \pm 11.59 b
Ethyl heptanoate	4.16 \pm 0.89 c	7.39 \pm 0.98 b	5.36 \pm 0.57 c	10.08 \pm 0.86 a
Ethyl caprylate	258.30 \pm 6.17 c	238.29 \pm 3.01 d	313.60 \pm 3.76 b	341.92 \pm 8.16 a
Ethyl decanoate	33.39 \pm 2.02 c	42.58 \pm 2.66 b	21.23 \pm 3.51 d	55.26 \pm 5.64 a
Ethyl benzoate	0.32 \pm 0.09 c	2.40 \pm 0.35 a	1.52 \pm 0.30 b	2.40 \pm 0.26 a
Diethyl succinate	8.51 \pm 0.49 b	5.81 \pm 0.25 d	6.59 \pm 0.60 c	9.66 \pm 0.22 a
Ethyl 9-decenoate	9.97 \pm 1.08 c	9.62 \pm 1.58 c	17.86 \pm 1.37 b	23.74 \pm 2.83 a
1,3-Propanediol, diacetate	21.29 \pm 0.66 c	21.69 \pm 0.75 c	26.65 \pm 2.09 b	31.33 \pm 1.48 a
Methyl salicylate	7.49 \pm 0.86 c	13.56 \pm 1.32 a	5.01 \pm 0.62 d	8.46 \pm 0.72 b

Etil-4-idrossibutanoato	21.35 ± 2.33 c	28.82 ± 1.06 b	43.56 ± 2.54 a	46.71 ± 3.37 a
2-Feniletacetato	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	8.83 ± 0.98 d	12.41 ± 1.03 c	21.00 ± 1.24 a	17.29 ± 1.79 b
Ethyl hydrogen succinate	27.24 ± 1.30 a	11.66 ± 1.02 c	15.29 ± 2.08 b	12.27 ± 1.89 bc
Ethyl vanillate	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	5.00 ± 0.63 c	5.45 ± 0.45 c	6.99 ± 0.62 b	11.67 ± 1.02 a
Isobutanol	212.83 ± 6.95 c	231.34 ± 4.06 b	228.02 ± 4.58 b	264.82 ± 10.36 a
<i>trans</i> -3-Penten-2-ol	7.28 ± 1.52 a	n.d.	9.04 ± 1.44 a	n.d.
Isoamyl alcohol	4403.08 ± 35.24 c	4411.70 ± 46.49 c	4865.19 ± 89.77 a	4601.17 ± 32.35 b
1-Pentanol	3.01 ± 2.02 a	4.97 ± 1.60 a	4.77 ± 1.98 a	4.98 ± 1.70 a
4-Methyl-2-pentanol	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	4.79 ± 0.61 b	6.16 ± 0.95 a	4.82 ± 0.47 b	7.18 ± 0.52 a
3-Methyl-1-pentanol	0.64 ± 0.25 b	0.99 ± 0.12 b	3.23 ± 0.68 a	3.68 ± 0.92 a
<i>n</i> -Hexanol	16.40 ± 2.01 d	25.23 ± 1.11 b	20.03 ± 1.51 cd	31.38 ± 1.83 a
3-Ethoxy-1-propanol	4.46 ± 0.84 b	4.62 ± 0.33 b	7.08 ± 0.33 a	7.87 ± 0.55 a
<i>cis</i> -3-Hexen-1-ol	6.91 ± 1.64 c	22.01 ± 1.56 a	4.37 ± 0.56 d	9.75 ± 0.82 b
1-Octen-3-ol	1.23 ± 0.90 b	8.82 ± 1.80 a	n.d.	6.48 ± 1.03 a
1-Heptanol	29.34 ± 1.97 b	31.19 ± 2.16 b	52.02 ± 3.16 a	53.22 ± 2.55 a
3-Methyl-2-octanol	2.57 ± 0.32 b	2.82 ± 0.24 ab	3.11 ± 0.28 a	3.26 ± 0.22 a
3-Nonanol	10.82 ± 0.86 ab	11.43 ± 1.08 a	9.59 ± 0.61 b	11.15 ± 1.00 a
2-Nonanol	7.15 ± 1.53 a	6.83 ± 1.40 a	6.72 ± 1.04 a	7.32 ± 1.85 a
2-3-Butanediol	341.27 ± 14.57 ab	315.57 ± 21.78 b	363.29 ± 11.78 a	361.10 ± 12.07 a
1-Ottanolo	12.06 ± 1.58 a	12.88 ± 2.11 a	11.90 ± 2.11 a	13.94 ± 1.54 a
Metionolo	17.23 ± 0.69 a	16.44 ± 0.77 a	17.83 ± 0.77 a	17.82 ± 0.91 a
Benzyl alcohol	17.10 ± 2.10 c	72.53 ± 3.97 a	17.64 ± 4.97 c	33.52 ± 6.85 b
Phenethyl alcohol	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	123.59 ± 3.79 b	115.93 ± 2.11 c	133.36 ± 2.73 a	135.61 ± 1.98 a
Propanoic acid	12.72 ± 1.24 b	16.95 ± 1.02 a	6.92 ± 1.11 d	9.11 ± 1.35 c
Isobutyric acid	64.27 ± 3.74 b	58.76 ± 3.47 b	75.18 ± 4.28 a	79.57 ± 4.15 a
Butanoic acid	24.02 ± 3.00 b	19.50 ± 4.72 b	26.45 ± 2.43 a	25.84 ± 2.81 a
Pentanoic acid	123.44 ± 7.76 b	108.74 ± 10.20 b	144.51 ± 9.38 a	155.00 ± 4.63 a
Hexanoic acid	1484.71 ± 35.48 a	1403.37 ± 82.21 a	1492.86 ± 47.56 a	1507.69 ± 122.08 a
(<i>E</i>)-2-Hexenoic acid	11.52 ± 2.66 c	31.76 ± 6.21 b	16.89 ± 3.01 c	45.08 ± 2.85 a
Caprylic acid	3309.91 ± 127.12 a	2810.37 ± 65.03 b	3136.37 ± 87.24 a	2810.10 ± 53.26 b
Nonanoic acid	10.50 ± 1.26 b	12.02 ± 1.58 ab	14.23 ± 1.75 a	15.00 ± 1.23 a
Capric acid	883.68 ± 36.82 a	445.95 ± 20.19 bc	405.69 ± 21.61 c	489.13 ± 36.15 b
9-Decenoic acid	206.82 ± 9.94 b	124.55 ± 7.39 c	240.96 ± 14.84 a	210.22 ± 11.72 b
Benzoic acid	84.44 ± 4.25 bc	77.97 ± 5.26 c	115.88 ± 14.53 a	91.62 ± 7.66 b
Lauric acid	180.00 ± 11.20 a	43.25 ± 6.24 c	81.18 ± 8.62 b	90.48 ± 7.00 b
Benzene acetic acid	220.93 ± 13.98 a	142.98 ± 14.12 b	226.53 ± 21.75 a	165.74 ± 9.01 b
Phenyl propionic acid	20.58 ± 2.49 bc	18.17 ± 1.13 c	30.41 ± 2.62 a	25.20 ± 1.83 b
Myristic acid	44.30 ± 2.08 a	14.65 ± 1.37 c	39.21 ± 3.66 a	27.23 ± 1.05 b
Palmitic acid	170.39 ± 10.73 b	99.05 ± 4.62 d	263.53 ± 15.82 a	140.55 ± 9.03 c
Stearic acid	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	n.d.	68.95 ± 2.32 a	0.09 ± 0.04 b	45.14 ± 7.12 b
6-Methyl-5-hepten-2-one	0.95 ± 0.17 c	2.64 ± 0.19 b	1.46 ± 0.47 c	4.27 ± 0.97 a
<i>cis</i> -Linalool oxide	0.96 ± 0.18 c	4.16 ± 0.32 a	1.98 ± 0.44 b	4.11 ± 0.28 a
<i>trans</i> -Linalool oxide	n.d.	1.06 ± 0.09 b	n.d.	1.98 ± 0.07 a
β-Linalool	66.86 ± 1.99 c	137.51 ± 1.32 a	73.71 ± 2.76 b	140.26 ± 2.40 a
<i>p</i> -Menth-2-en-1-ol	0.22 ± 0.02 b	0.41 ± 0.12 a	0.21 ± 0.03 b	0.47 ± 0.09 a
β-Caryophyllene	8.41 ± 0.77 c	16.85 ± 1.75 a	13.60 ± 0.92 ab	15.29 ± 0.78 a
Terpinene-4-ol	1.74 ± 0.44 b	4.19 ± 0.62 a	1.87 ± 0.66 b	3.62 ± 0.32 a
(<i>E</i>)-Farnesene epoxide	10.84 ± 1.14 c	21.10 ± 1.90 b	12.05 ± 0.78 c	29.19 ± 2.10 a
α-Humulene	2.36 ± 0.72 c	21.63 ± 1.89 a	3.14 ± 0.64 c	18.88 ± 1.15 b
α-Terpineol	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	2.05 ± 0.21 b	5.22 ± 0.82 a	2.56 ± 0.78 b	7.14 ± 1.64 a

β -Citronellol	20.75 \pm 1.50 c	63.88 \pm 7.48 a	19.37 \pm 1.11 c	38.47 \pm 3.21 b
<i>cis</i> -Geraniol	5.49 \pm 0.70 d	14.45 \pm 1.31 a	8.51 \pm 0.34 c	10.95 \pm 0.99 b
<i>trans</i> -Geraniol	0.30 \pm 0.03 b	0.60 \pm 0.11 a	0.60 \pm 0.06 a	0.67 \pm 0.04 a
<i>p</i> -Mentha-1,8-dien-7-ol	16.01 \pm 1.46 d	82.11 \pm 1.46 a	37.56 \pm 1.21 c	61.00 \pm 2.54 b
8-Hydroxy linalool	10.33 \pm 1.80 c	25.59 \pm 1.98 a	13.59 \pm 1.63 bc	16.50 \pm 1.25 b
Farnesol	21.78 \pm 1.09 b	22.87 \pm 1.89 b	30.89 \pm 2.26 a	29.00 \pm 2.20 a
Dihydro- β -ionone	12.41 \pm 2.86 c	67.02 \pm 3.71 a	52.87 \pm 3.01 b	54.90 \pm 4.25 b
Total Terpenes	587.23 \pm 35.28 c	1.006.18 \pm 42.22 a	585.61 \pm 29.28 c	832.44 \pm 45.69 b
1,1-Dimethyl-4-methylenehexahydro-1H-cyclopenta[c]furan	n.d.	3.86 \pm 0.35 b	n.d.	7.03 \pm 0.64 a
Furfuryl alcohol	11.11 \pm 0.70 ab	10.72 \pm 0.46 b	12.19 \pm 1.09 a	10.84 \pm 0.64 b
5-Ethylidihydro-2(3H)-furanone	2.77 \pm 0.48 a	3.20 \pm 0.23 a	3.23 \pm 0.34 a	2.73 \pm 0.45 a
2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	23.03 \pm 1.11 a	21.00 \pm 1.32 ab	20.50 \pm 0.52 b	20.31 \pm 0.93 b
2,3-Dihydro-benzofuran	31.43 \pm 2.72 b	47.63 \pm 3.23 a	25.83 \pm 2.07 c	31.36 \pm 2.39 b
Total Furans	68.34 \pm 5.02 bc	86.41 \pm 5.58 a	61.75 \pm 4.02 c	72.27 \pm 5.05 b
2-Methoxy-4-vinylphenol	99.60 \pm 5.17 b	108.26 \pm 4.17 b	139.63 \pm 2.25 a	143.52 \pm 2.26 a
Acetovanillone	33.81 \pm 1.98 c	34.64 \pm 1.18 c	42.86 \pm 1.02 b	51.74 \pm 3.25 a
Methoxy-eugenol	17.25 \pm 0.95 c	20.09 \pm 1.01 b	21.22 \pm 1.02 ab	24.80 \pm 2.28 a
<i>trans</i> -Cinnamic acid	72.39 \pm 0.99 a	69.22 \pm 2.07 a	63.99 \pm 1.45 b	59.19 \pm 2.41 b
Tyrosol	155.29 \pm 10.57 b	132.12 \pm 9.09 c	208.95 \pm 13.58 a	145.30 \pm 11.56 bc
Total Phenols	378.35 \pm 19.66 c	364.34 \pm 17.51 c	476.66 \pm 19.33 a	424.55 \pm 21.76 b
Methyl acetoin	4.10 \pm 0.38 a	3.79 \pm 0.68 a	4.29 \pm 0.74 a	4.52 \pm 0.38 a
Acetoin	45.84 \pm 4.31 a	10.27 \pm 1.24 c	15.47 \pm 1.23 b	17.19 \pm 1.20 b
3-Hydroxy-3-methyl-2-butanone	14.03 \pm 1.01 a	12.07 \pm 0.67 b	13.23 \pm 0.50 ab	13.56 \pm 0.58 a
γ -Nonalactone	35.83 \pm 0.73 b	36.42 \pm 0.74 b	47.07 \pm 1.22 a	45.13 \pm 1.56 a
Total Ketones	99.80 \pm 6.43 a	62.54 \pm 8.92 c	80.06 \pm 7.71 b	80.41 \pm 8.78 b
<i>n</i> -Nonanal	2.20 \pm 0.35 ab	1.97 \pm 0.21 b	1.93 \pm 0.15 b	2.59 \pm 0.24 a
Total Aldehydes	2.20 \pm 0.35 ab	1.97 \pm 0.21 b	1.93 \pm 0.15 b	2.59 \pm 0.24 a
2-Acetylpyrrole	47.98 \pm 1.82 a	40.24 \pm 2.22 b	39.04 \pm 2.82 b	40.46 \pm 1.22 b
5-Methyl-1H-pyrrole-2-carboxaldehyde	7.15 \pm 0.60 b	8.68 \pm 0.35 a	7.45 \pm 0.93 b	7.98 \pm 0.39 ab
Styrene	n.d.	n.d.	14.68 \pm 2.09 a	12.62 \pm 1.86 a
Total Others	55.13 \pm 2.43 b	48.91 \pm 2.57 c	61.18 \pm 3.76 a	61.06 \pm 3.47 a

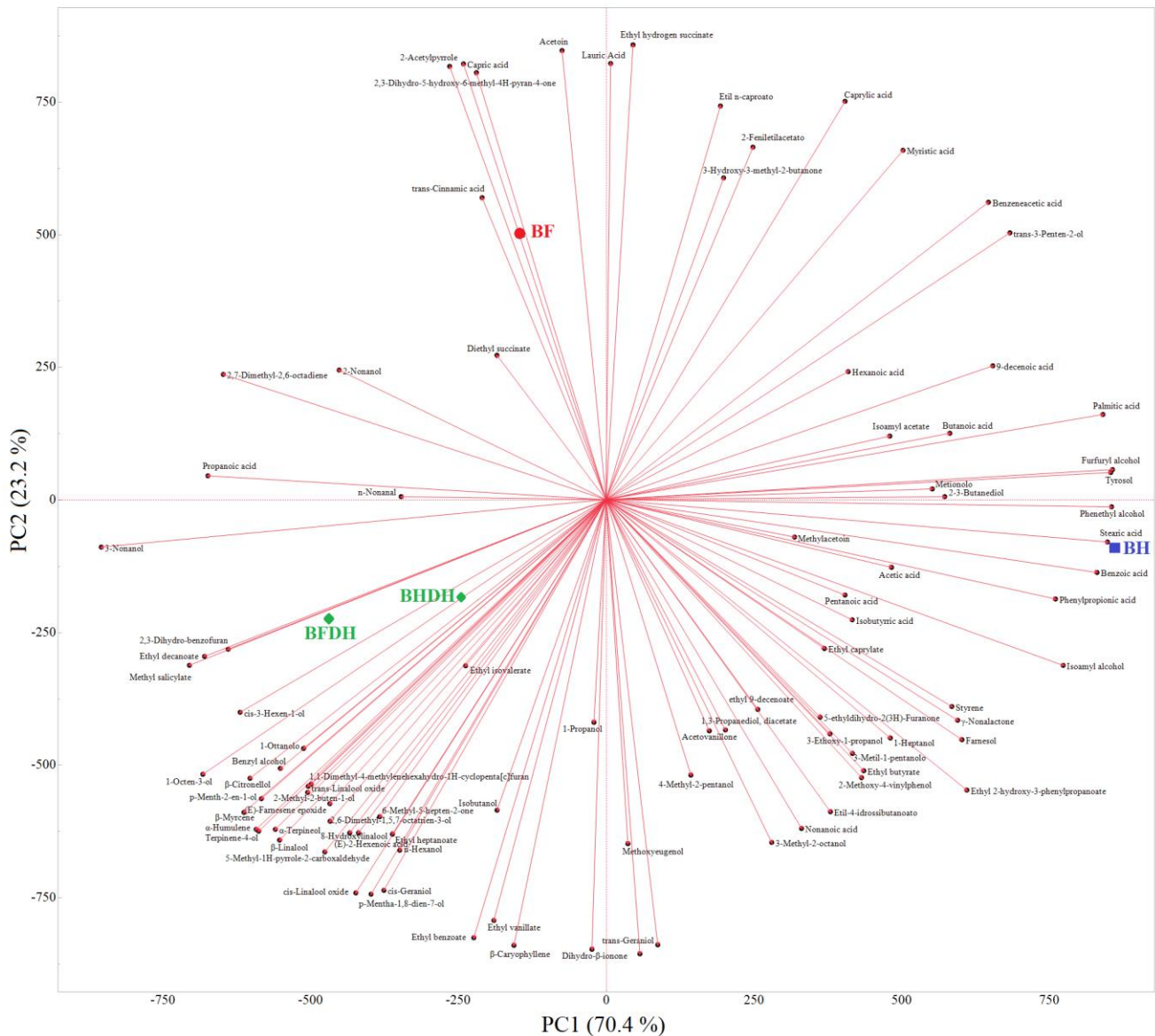
212 Different letters in each row refer to significant differences (Tukey, $p < 0.05$). n.d.= not detected.

213 As regards VOCs (Table 3), during fermentation and maturation different species of secondary compounds originate, which char-
214 acterize the sensory profile of beer. Esters provide beer with fruity and floral scents (Brányik et al., 2008); we found mainly isoamyl
215 acetate especially in BH samples and very low concentration in BFDH (BH 450.60 μ g/L; BHDH 478.83 μ g/L; BF 361.63 μ g/L;
216 BFDH 130.87 μ g/L); also ethyl caprylate was higher in BH samples while ethyl-*n*-caproate was the highest in BF but the lowest
217 in BFDH. Concentrations of isoamyl acetate above 2 mg/L give fruity scents (olfactory threshold 1.2 ppm) (Olaniran et al., 2017),
218 while ethyl caprylate and ethyl-*n*-caproate provide aromas of acidic apple in concentrations higher than 0.9 ppm and 0.21 ppm,
219 respectively (Kobayashi et al., 2007); in our samples the content detected was below the olfactory perception threshold. Totally
220 speaking, in freeze-dried hop, using the dry-hopping reduced the amount of esters while in stove hop, the dry-hopping increased the
221 esters. Alcohols were ten-fold higher than esters and the total highest concentration was in BH (Table 3). Among the higher alcohols
222 the most important in term of concentration were isoamyl alcohol (BH 4865.19 μ g/L; BHDH 4601.17 μ g/L; BF 4403.08 μ g/L;
223 BFDH 4411.70 μ g/L) and 2-phenylethyl alcohol (BH 8645.00 μ g/L; BHDH 7608.98 μ g/L; BF 7740.16 μ g/L; BFDH 7524.38 μ g/L).
224 Isoamyl alcohol if present in high concentrations negatively affects the drinkability of the product (Olaniran et al., 2017); however,
225 the quantity detected in the test samples was below the limit at which this adverse effect is observed. 2-phenylethyl alcohol, which
226 would give hints of rose, peppermint and orange blossom, was also below the threshold of perception (125 ppm) (Olaniran et al.,
227 2017). The highest concentration of these two alcohols was anyway in BH sample. Caprylic acid was in the greatest amount among
228 acids, especially where dry-hopping was not used, followed by hexanoic acid (Table 3). The total amount of acids was higher where

229 dry-hopping was not used. These acids are from cell membrane thus coming from yeast degradation and are important in the for-
230 mation of non acetic esters which play an important role for the fruity aroma. Add of dry-hopping in freeze-dried beer reduced
231 significantly acids and consequently esters, reducing the aromatic potential. The reason of this significantly reduction especially in
232 BF samples could be due to the presence of other microorganisms which are survived after the freeze-drying process and could have
233 used to built cell membrane and reproducing (Romero-Rodríguez et al., 2022).

234 In general, hops strongly contribute to the sensory characteristics of the product, through α - and β -acids, and to the aromatic ones,
235 depending on the concentration of terpenes (Hauser et al., 2019; Lafontaine & Shellhammer, 2018). In the class of terpenes, 20
236 compounds were identified (Table 3). The drying methodology determined a different influence on the aromatic component of the
237 beer, also related to the different infusion technique, as evidenced by the hierarchical cluster shown in Figure 1. Beers produced
238 with the dry-hopping technique, had a significantly higher content in terpenes especially in beer from freeze-dried hops. 2,7-
239 Dimethyl-2,6-octadiene was in significantly higher concentration than the other compounds usually characterizing hop (Mastrangelo
240 et al., 2023; Steyer et al., 2017) but the difference between dry-hopping and not, was marked by β -linalool and β -citronellol in
241 double concentration in BFDH and BHDH samples. The values here reported are much higher than what is present in the literature
242 (Van Opstaele et al., 2010). Freeze-drying and dry-hopping gave the highest concentration of these aromatic compounds confirm-
243 ing the role of hops in providing terpenes which are preserved by using the least invasive drying technique. Total furans were higher
244 in BF samples while total phenols in BH samples, regardless dry-hopping.

245 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
246 %), while the remaining three PCs explain the residual variance (PC3 6.4 %). Therefore, we have only reported the first 2 PCs. The
247 PCA of Volatile Organic Compounds (VOCs) clearly distinguishes the beer without dry-hopping using the two different techniques.
248 Specifically, they are positioned in opposite directions in the 2nd and 4th quadrants, denoted as BF and BH, respectively. Interest-
249 ingly, the dry-hopped beers, regardless of the type of hops used, are clustered together in the 3rd quadrant. This observation could
250 be attributed to the technique, wherein the second cold hopping phase may lead to the release of certain compounds (such as ter-
251 penes) or an increase in others do not present in beers without dry-hopping (BF and BH).

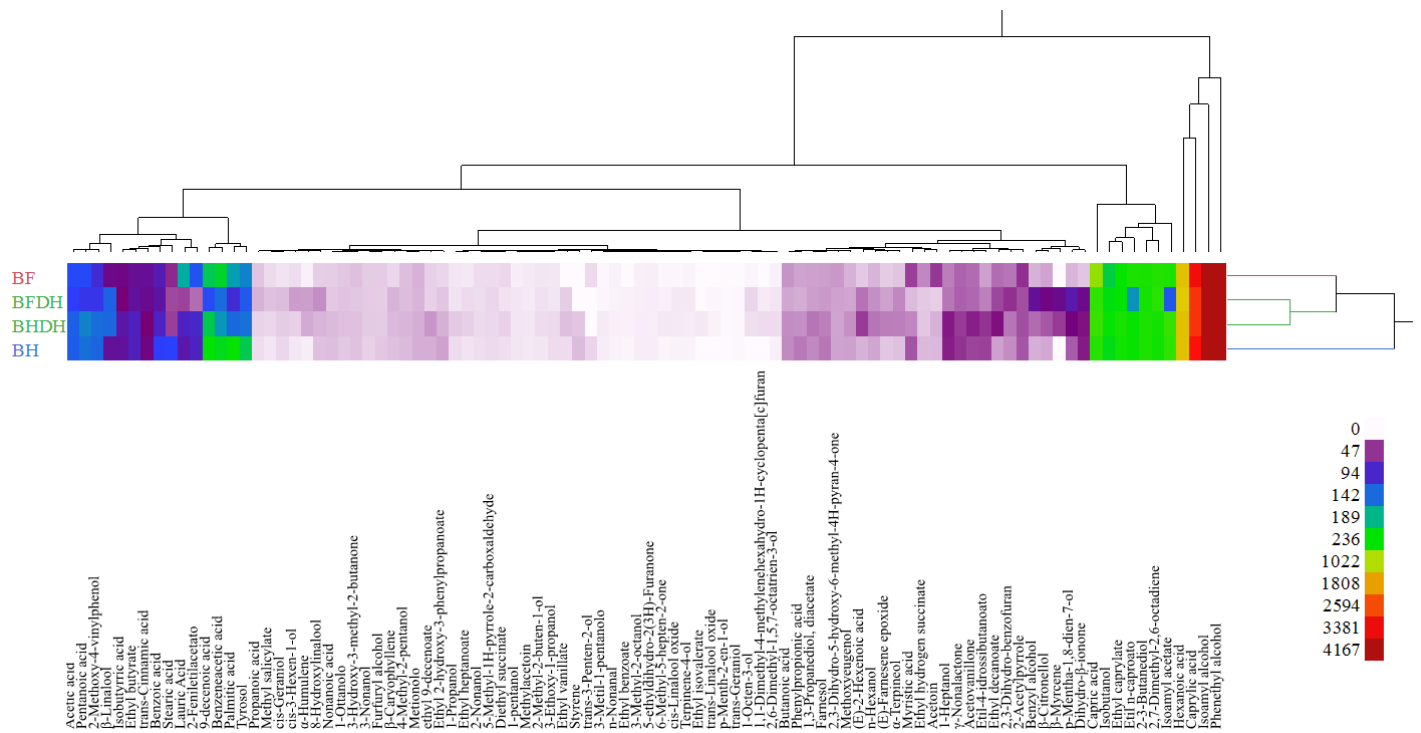


252

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

257 The data of the PCA, has also confirmed by the hierarchical cluster analysis (HCA) reported in Figure 2. The HCA showed how the
 258 two beers with the dry-hopping (BHDH and BFDH) cluster together and have a profile separated to the other as confirmed the
 259 strong effect of this techniques on the aromatic profile of beers conferring a greater complexity profiler to the product such as terpene
 260 compounds.

261 The beer without dry-hopping (BF and BH), are clearly separated in two different cluster, and this is correlated to the techniques of
 262 drying of hop (F and H) which produce different aromatic compounds in the product, probably related to the drying temperature
 263 used.

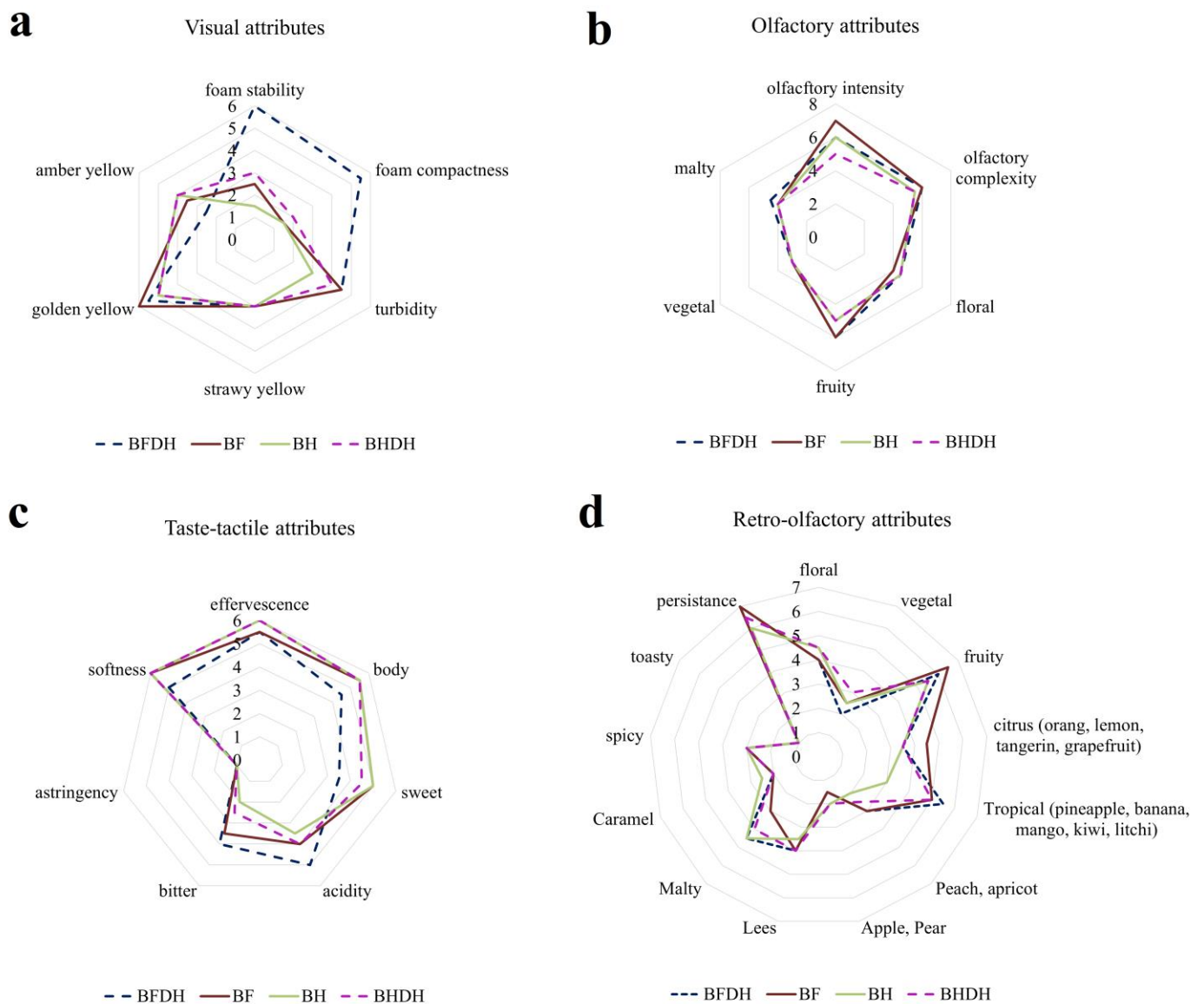


264

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

268 **3.3 Sensory evaluation**

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



271

272 **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).

276 As general consideration it can be stated that very low scores for vegetal, apple and pear, caramel and toasty notes are common for all the 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.

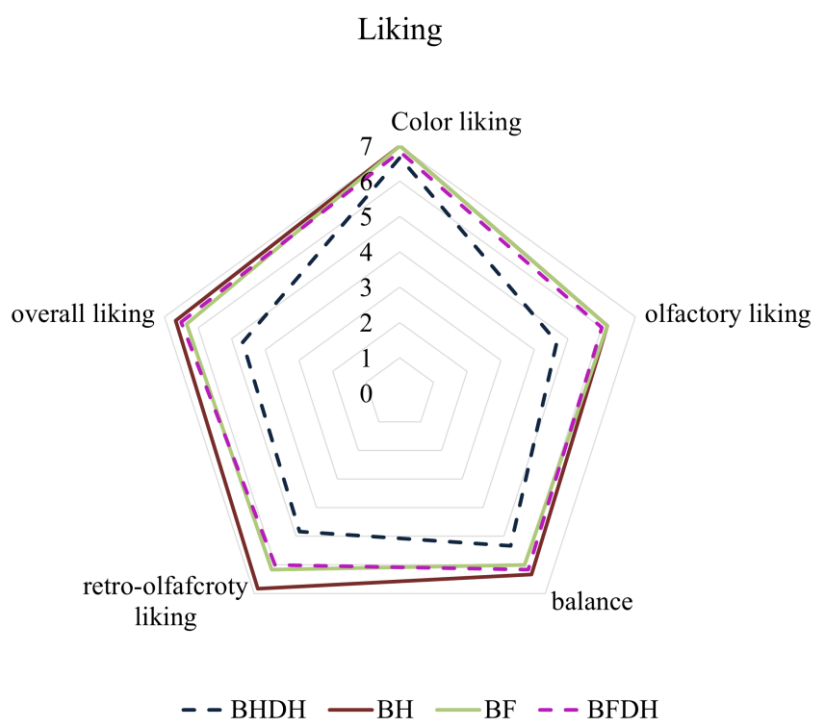
279 Considering BF sample, it was described as golden yellow with amber components and a present turbidity, foam was neither stable nor compact. The aroma profile at direct olfaction was the most intense and complex of the set characterized by a predominant fruity note. Together with most of the other samples, it was considered soft, medium effervescent, with a good sensation of body and sweetness. Acidity and bitterness were low, the same for the astringency. What already said for the aroma found confirmation in the retro-olfactory perception: it is persistent fruity and citrus notes prevailing upon the others.

284 Moving to the dry-hopped version BFDH resulted in more golden than amber, picked for foam compactness and stability and was as turbid a BF. The olfactory profile was less intense but as complex as the previous characterized by floral, fruity and malty notes. Taste wise this BFDH was the most acidic and bitter sample, this not only because of the composition but also because of lack of

287 body, softness, and sweetness able to counterbalance these perceptions. The flavor profile was long as for BF and characterized by
288 tropical fruits, malt, and lees scents.

289 The two beers produced with hot-stoved hop were the more ambered and least golden in color, with BH that was the poorest for
290 foam stability and compactness, but also the less turbid. BHDH was comparable to BF in terms of foam and turbidity. In respect of
291 the olfactory analysis, these two samples showed lower complexity and, particularly BHDH, also lower intensity, with a bouquet
292 characterized by more floral than fruity notes. Considering mouth perception, hot-stove hop beers were the most effervescent and
293 soft with the lower bitterness and, considering BH sample, the lower acidity. Considering body, softness and sweetness these two
294 samples are aligned with BF samples. The retro-olfactory profile is less complex than those for freeze-dried hop beer, more vegetal
295 and lees like for BHDH and more malty and caramel like for BH. Apple and pear, even if at low level, were perceived as flavors of
296 these beers.

297 Moving to the liking, the least liked beer was BHDH for all the different aspects; the other three samples were generally liked
298 evenly, with BH picking for retro-olfactory liking and balance (Figure 4).



299

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

303 4. Conclusions

304 The research activity carried out showed a clear demarcation, both aromatic and sensory, between the beers produced with different
305 hop drying technique. Hops dried with hot stove produced beer with high ethanol and titratable acidity and low in volatile acidity.
306 Color of the beer was lighter using hot-stove dried hops due to the polyphenol oxidation occurred in hop freeze dried. Dry-hopping
307 has emerged as a powerful tool for brewers to modify the sensory profile and overall quality of beer. Through the addition of hops
308 during fermentation or post-fermentation, brewers can enhance aroma, flavor, and consumer satisfaction. Beers produced with the
309 dry-hopping technique, had a significantly higher content in terpenes especially in beer from freeze-dried hops. BFDH resulted in
310 more golden than amber, picked for foam compactness and stability and was as turbid a BF. The olfactory profile was less intense

311 but as complex as the previous characterized by floral, fruity and malty notes. Taste wise this BFDH was the most acidic and bitter
312 sample. Finally, this paper highlighted the chemical changes which occurred between drying techniques and using hop-drying and
313 we cannot say what it was better because it depends on the consumer taste. We emphasized that beers were different in chemical
314 features, VOCs content and diversity, and in sensory analysis but none of them showed off-flavor and off-odor.

315 **CRedit author statement**

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

324 **Declaration**

325 **Competing Interest:** The authors declare that they have no known competing financial interests or personal relationships that could
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327 **Data availability:** Data will be made available on request.

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462

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:

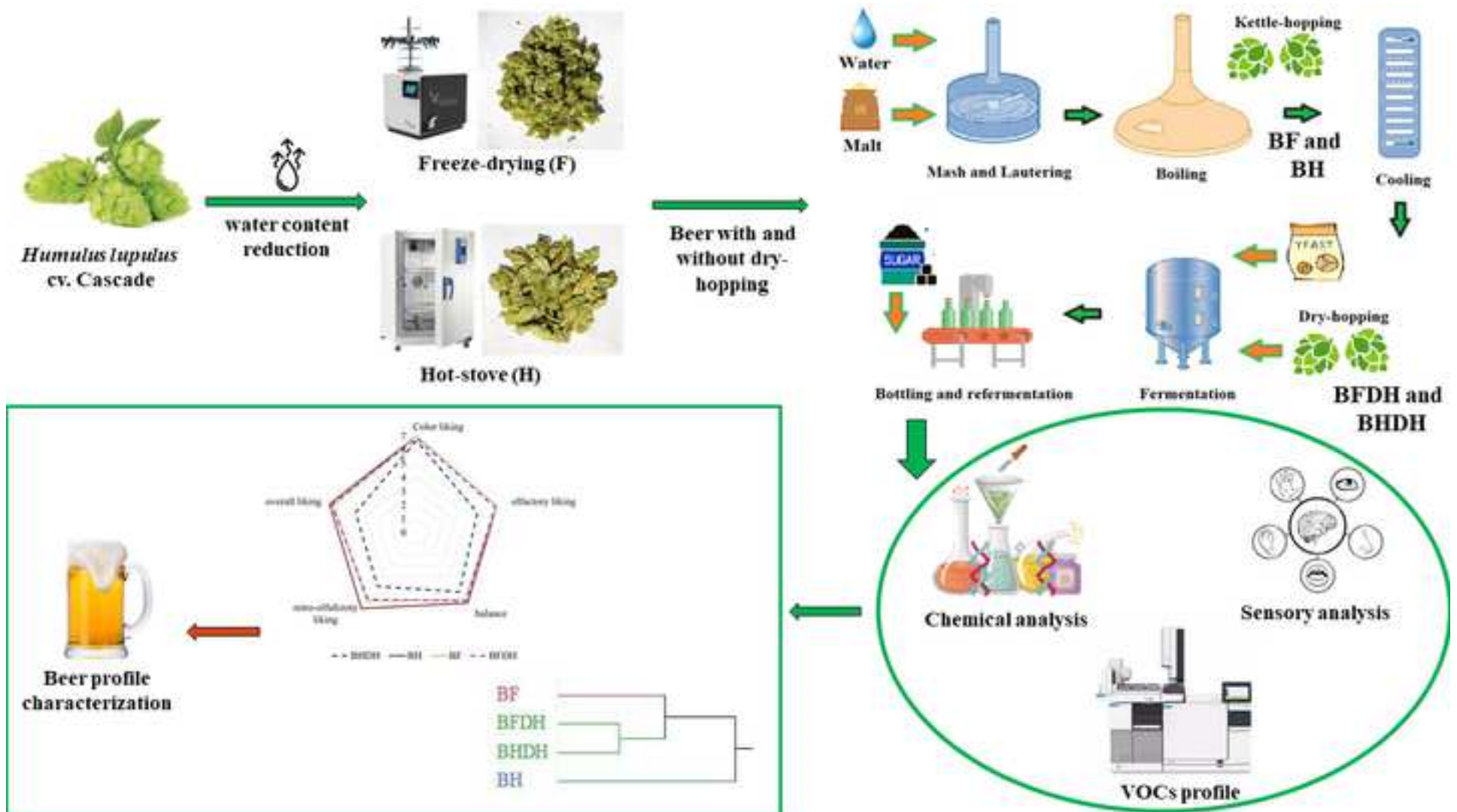


Figure 1

