From Hops to Craft Beers: Production Process, VOCs Profile Characterization, Total Polyphenol and Flavonoid Content Determination and Antioxidant Activity Evaluation

Matteo Iannone 1, Elisa Ovidi 2, Sara Vitalini 3,4,5, Valentina Laghezza Masci 2, Andrea Marianelli 1, Marcello Iriti 3,4,5,6, Antonio Tiezzi 2 and Stefania Garzoli 7,*

1 Circolo ARCI La Staffetta, 56011 Pisa, Italy; arcilastaffetta@gmail.com (M.I.); andreamarianelli93@gmail.com (A.M.)
2 Department for the Innovation in Biological, Agrofood and Forestal Systems, Tuscia University, 01100 Viterbo, Italy; ovidi@unitus.it (E.O.); laghezzamasci@unitus.it (V.L.M.); antoniot@unitus.it (A.T.)
3 Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milan, Italy; sara.vitalini@unimi.it (S.V.); marcello.iriti@unimi.it (M.I.)
4 Phytochem Lab, Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milan, Italy
5 National Interuniversity Consortium of Materials Science and Technology (INSTM), Università degli Studi di Milano, 20133 Milan, Italy
6 Center for Studies on Bioinspired Agro-Environmental Technology (BAT Center), Università degli Studi di Milano, 20133 Milan, Italy
7 Department of Drug Chemistry and Technology, Sapienza University, 00185 Rome, Italy
* Correspondence: stefania.garzoli@uniroma1.it

Abstract: In this work, selections of seven international hop varieties and three craft beers obtained from them were analyzed by SPME-GC/MS techniques with the aim to describe their volatile chemical profile. The brewing process was also reported. Furthermore, the hop extracts and beers were investigated to determine their flavonoid and polyphenol content and to evaluate their antioxidant power by DPPH and ABTS assays. The findings showed the presence of compounds belonging to different chemical classes such as monoterpenes, sesquerpenes, alcohols, esters and fatty acids. In particular, sesquerpenes were the main compounds with β-caryophyllene (from 1.7 to 16.2%) and humulene (from 10.8 to 43.9%) as the major components in all varieties of dried hop cones investigated. On the contrary, with the exception for the Pacific sample, monoterpenes were the class of compounds that were more abundant in the hop extracts and, among these, β-myrcene appeared to be the predominant constituent (from 31.8 to 71.4%). Regarding the craft beers obtained by adding these hop varieties, some differences in the qualitative and quantitative volatile composition have been found. All hop samples showed a high scavenging potential against both radicals. In the case of DPPH, the obtained IC 50 values ranged from 0.027 to 0.047 mg/mL while they varied between 0.023 and 0.134 mg/mL by the ABTS assay. A positive correlation was found with polyphenol and flavonoid contents. Among beer samples, ACD was the richest one in polyphenols (292.0 mg GAE/100 mL beer) and flavonoids (5.8 mg QE/100 mL beer) and the most powerful against DPPH•− and ABTS•+ radicals with IC 50 values equal to 4.969 and 0.198 v/v%, respectively.

Keywords: Humulus lupulus; beer; SPME-GC/MS; volatile compounds; ABTS assay; DPPH assay

1. Introduction

Hops (Humulus lupulus L.) belongs to the Cannabaceae family and its cultivation is widespread all over the world. It is a dioecious, perennial and climbing plant whose female inflorescences are used to produce beer, and they attribute the bitter taste while maintaining its stability over time [1]. Female inflorescences (also called hop cones or simply hops) are grown almost exclusively for the brewing industry and, from a chemical point of view,
are characterized by a complex pool of secondary metabolites including a terpene fraction responsible for the characteristic fragrance of the produced beers. Furthermore, hop cones have long been used for medicinal purposes; in fact, given the effects on the central nervous system, they were especially helpful in the regulation of sleep disorders [2]. Several studies report the multiple activities of hops too as anticancer agents [3–8].

The brewing process as well as climatic and ecological conditions of hops are very important for defining the characteristic flavor and taste of the beer. Beer is one of the most consumed drinks in the world, and Germany and the USA are the countries that produce the largest quantities of beer equal to about 80% of total production [9]. Although there are several varieties, the production process involves the fermentation of malt extracts from barley and other cereals with possible additions of flavoring agents such as hops, various herbs and fruits. In particular, hops provide bitterness and aromas (odor) and also induce a preservative effect thanks to its antibacterial activity. The final product is very rich in volatile organic compounds (VOCs), which are responsible for aroma and taste, hence the interest in the volatile chemical characterization of beers.

To our knowledge, this is the first paper that reports the volatile chemical composition investigated by SPME-GC/MS of seven varieties of dried hop cones (STG: Styrian Goldings; HBK: Hersbrucker; HLB: Hallertau Blanc; CCH: Cascade CryoHop; PCJ: Pacific Jade; PC: Pacifica; TP: Topaz) and of the three beers (IPA: Ipagea; LKO: Lokomotiv; ACD: Accademica), as final products describing the brewing process used for this purpose. Furthermore, additional chemical analysis was carried out on methanol extracts (STGe: Styrian Goldings; HBKe: Hersbrucker; HLBe: Hallertau Blanc; CCHe: “Cascade Cryo Hops®”; PCJe: Pacific Jade; PCe: Pacifica; TPe: Topaz) tested for the determination of their polyphenolic and flavonoid content and of the antioxidant power.

2. Materials and Methods

2.1. Materials

Hop pellets were purchased from Mr. Malt® P.A.B. S.R.L. Via Moretti 4 33037—Pasian di Prato (UD) Italy by Yakima Chief Hops, LLC.

Methanol, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diaminonitrat salt (ABTS), potassium persulfate (K₂S₂O₈), Folin & Ciocalteu’s phenol reagent, sodium carbonate, aluminum chloride, gallic acid, potassium acetate and quercetin were purchased from Merck (Darmstadt, Germany)

2.2. Brewing Process

The grist (ground malt grains) recipes for Lokomotiv (LKO—Blanche beer) was composed of 75.0% of Pilsner as base malt and 25.0 % flaked wheat for foam and sourish profile. Accademica (ACD—Belgian Ale) comprised 92.5% of Dingemans Pale Ale Belgian base malt, with the addition of 7.5% of aromatic malt for more color and flavor. Ipagea (IPA—White Ipa) comprised 75.0% of Pilsner and 15.0% of Weizen as base malt, 3.0% Carapils malt for more body, 10.0% flaked (wheat and oat) and 2.0% acid malt for lower acidity of beer wort. The mashing process of the ground grains (grains:water in a 1:3 ratio) was performed in a multistep system. Once the mixture had reached 45 °C, the temperature program proceeded as follows:

(1) 45 °C for 10 min: IPA, LKO and ACD beer samples (protease enzymes react to hydrolyze low-weight protein as nourishment for yeast);
(2) 50 °C for 20 min: IPA and LKO beer samples (amylolytic activity);
(3) 62 °C for 20 min: IPA and LKO beer samples (β-amylase activity, pH 5.0–5.5, maximum activity);
(4) 66 °C for 20 min: IPA, LKO and ACD beer samples (β-amylase activity, pH 5.0–5.5, enzymatic synergy point between amylases);
(5) 72 °C for 20 min: IPA beer sample (α-amylase activity, pH 5.6–5.8, maximum activity);
(6) 78 °C for 5 min: IPA, LKO and ACD beer samples (enzymatical inactivation phase).
After 15 min of cooling, filtering took place with the washing of the threshes and the collection of the wort in a sanitized fermenter; this process was repeated 6 times with water at pH 6. The wort boiling phase was performed for 1 h (for all samples), together with bitter and aroma hopping. The type of hops used for each recipe is listed below (Table 1).

| Table 1. Type of used hops. |
|---|---|---|
| **Hops** | Topaz | Pacific Jade |
| **Organoleptic characteristics** | Light, tropical fruit flavors of lychee | Flavors of lemon citrus and cracked pepper |
| ** Purpose** | Aroma | Bittering and Aroma |
| **Country** | New Zealand | USA |

The hop pellets were used mostly for aroma attributes: they were added in the last 0–10 min of the boiling phase to transfer scent and aroma.

The beer wort was then cooled during the whirlpooling phase with a counter flow heat exchanger. The cooling phase was performed using a plate-heat exchanger, where the hot mash and coolant (tap water) circulate in opposite directions. The mash was then oxygenated to favor the beginning of the fermentation, stirring for at least a couple of minutes. Finally, yeasts (Fermentis SafAle™ US-05, Lesaffre, Cedex, France) were inoculated and the mix was stirred again. The mix was closed in the fermenter for 12 days at 20 °C, with a gradual temperature decrement down to 4 °C.

Then, bottling and priming processes were performed: the bottles were stored at 22–25 °C for 20 days; then, the nucleation of carbon dioxide was repeated by placing the bottles in a refrigerator at 4 °C for 4–5 days.

2.3. Extract Preparation

Ten grams of each H. lupulus hop samples was placed in flasks and extracted under stirring in 100 mL of methanol for 2 h at RT. The mixtures were filtered by filter paper (Whatman n. 2) and then by a filter syringe (0.22 μm). The solvent was evaporated in a rotary evaporator (RV 08-VC, IKA, Staufen, Germany), and the residues were stored into glass vials at 4 °C until use.

2.4. SPME Sampling

To obtain the volatile chemical composition of the hop inflorescences and beers, the sampling was carried out using SPME technique following Vitalini et al. [10] with some modifications. Small amounts of inflorescences (~2 g) and beer (~2 mL) were individually placed into a 20 mL glass vial with PTFE-coated silicone septum. For the extraction of volatiles, an SPME device from Supelco (Bellefonte, PA, USA) with 1 cm fiber coated with 50/30 μm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) was used.

Before use, the fiber was conditioned at 270 °C for 30 min. All samples of beer and inflorescences were equilibrated for 20 min at 50 °C before sampling. Later, the fiber was exposed to the headspace of the samples for 30 min 50 °C to collect and concentrate volatiles compounds. Lastly, the SPME fiber was inserted in the GC injector maintained at 250 °C in split mode for the desorption of captured components.

2.5. GC-MS Analysis of Hop Inflorescences and Beers

The chromatographic analyses of the headspace from inflorescences and beers were carried out on Clarus 500 model Perkin Elmer (Waltham, MA, USA) gas chromatograph coupled with a mass spectrometer equipped with an FID (flame detector ionization) and
a Varian Factor Four VF-1 (60 m × 0.32 mm, 1.0 μm of film thickness) capillary column. The oven temperature was programmed initially at 60 °C and then increased to 220 °C at 6°/min and finally held for 15 min. Helium was used as a carrier gas at a constant rate of 1 mL/min. The mass spectrometer was operated at 70 eV (EI) in scan mode in the range 40–400 m/z. Ion source and the connection part temperatures were 220 °C.

The identification of volatile compounds was performed by matching their mass spectra with those stored in the Wiley 2.2 and Nist 02 mass spectra libraries database and by calculating the Linear Retention Indices (LRIs) by using a series of alkane standards analyzed under the same conditions as that of the samples. LRIs were then compared with available retention data reported in the literature. The peak areas of the FID signal were used to calculate the relative quantities of the components expressed as percentage without the use of an internal standard and any factor correction. All analyses were carried out in triplicate.

2.6. GC-MS Analysis of Extracts

Each methanol extract measuring 1 μL was injected manually at 270 °C into the GC injector, and the injector split ratio was 1:20. Analyses were carried out according to the following experimental conditions: initially at 60 °C then increased to 170 °C at 4°/min, then increased to 250 °C for 3 min at 5°/min and finally held for 15 min. The mass spectrometer operated at the same conditions reported above and the identification and quantification of the compounds were performed as previously described.

2.7. Determination of Total Phenolic Content

The total phenolic content was determined by the Folin–Ciocalteu method following Vitalini et al. [10]. Briefly, a mixture with an adequate amount (2.5 or 5 μL) of each sample (beer or hop extract) and 0.05 mL of the Folin–Ciocalteu reagent placed in a 10 mL test tube was kept at room temperature for 3 min. Then, 0.1 mL of a saturated sodium carbonate solution was added and a final volume of 2.5 mL was achieved with distilled water. The reaction was allowed to continue in the dark for 60 min, after which absorbance was measured at 765 nm with respect to a blank using a Jenway 6310 spectrophotometer (Keison, Chelmsford, Essex, UK). The test was performed in triplicate three times and the results reported as milligram of Gallic Acid Equivalents (GAE) per 100 mL of beer or per gram of dry hops.

2.8. Determination of Total Flavonoid Content

The total flavonoid content was determined by the aluminum chloride colorimetric method following Vitalini et al. [10]. Briefly, 0.5 mL of each suitably diluted sample was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The final solution was incubated in the dark for 30 min at room temperature. Then, absorbance was measured at 420 nm using a Jenway 6310 spectrophotometer (Keison, Chelmsford, Essex, UK). The test was performed in triplicate three times, and the results are reported as milligram of Quercetin Equivalents (QE) per 100 mL of beer or per gram of dry hops.

2.9. Antioxidant Assays

To determine the radical scavenging activities of the analyzed samples, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt assays were used. All hop-dried extracts were diluted in methanol (1 mg/mL) while beer samples were tested as is.

As detailed by Ovidi et al. [11], a DPPH assay was carried out, and the absorbances of eleven geometric dilutions in methanol of each sample, Trolox and the corresponding controls were measured at 517 nm.

For the ABTS assay, a working solution was prepared by mixing the ABTS solution (7 mM) in acetic acid buffer and the potassium persulfate (2.45 mM) solution for 16 h
before use to allow compounds to form the ABTS•+ radical cation. The working solution was then diluted in acetate buffer until the absorbance was 0.74 ± 0.03 at 734 nm. To test antioxidant activity, 10 µL of each sample was added to 1990 µL of working solution, and after 5 min, the absorbances were measured at 734 nm by a Jasco V-630 UV-Visible spectrophotometer (JASCO Europe, Lecco, Italy) and operating Spectra Manager™ software. Trolox geometrical dilutions were used as positive control and data were obtained in triplicate for each sample.

For both assays, the percentage of the antioxidant activity (A.A%) was calculated using the following formula:

$$\%\text{A.A.} = \left( \frac{A_D - A_S}{A_D} \right) \times 100$$

where %A.A. is the percentage of antioxidant activity, $A_D$ is the absorbance of blank control and $A_S$ is the absorbance of the sample [12].

IC$_{50}$ values of the samples (mg/mL for hop samples and %v/v for beer samples) and Trolox (mM) were calculated using calibration curves by plotting %A.A. against sample concentrations.

For both assays, the values, expressed in Trolox equivalent (TEAC), were obtained from the ratio between Trolox IC$_{50}$ and sample IC$_{50}$ (µmol of Trolox equivalent/milligram of dry hop samples) [13].

2.10. Statistical Analysis

All data were expressed as means ± standard deviation (SD). Statistical analysis was preformed using one-way ANOVA test with a Stat-Plus software (AnalystSoft VC 2009) with the threshold of significance set at $p < 0.05$.

3. Results

3.1. Vapor Phase H. lupulus Dried Cone Chemical Composition

By SPME-GC/MS analysis, the volatile composition of dried hops was described. In total, forty components were identified, and they are listed in Table 2. Except for Hd6, monoterpenes were the class of compounds that were more abundant and, among these, β-myrcene was the major component in all samples (from 31.8% to 71.4%). Other minor compounds such as β-thujene, β-pinene, limonene, cis-β-ocimene and linalool were detected with values between 0.1% and 0.8%. Humulene was the major sesquiterpene and the higher percentage mean value was found in PC hop (43.9%). β-Caryophyllene, as the second most abundant component, reached similar values in STG (16.2%) and in PC (15.1%) hops. Moreover, β-eudesmene was present in all hop samples albeit with lower quantities (from 0.2% to 3.6%). Differences in the qualitative composition of hop varieties have been found; in fact, some sesquiterpene compounds were detected only in some hop samples and, in particular, α-selinene, which in HLB hop reached 11.8%, was missing in all others.
### Table 2. Chemical volatile composition (percentage mean value ± standard deviation) of dried hops by SPME-GC/MS analysis.

| N° | Component                  | LRI 2 | LRI 3 | STG(%) | HBK(%) | HLB(%) | CCH(%) | PCJ(%) | PC(%) | TP(%) |
|----|----------------------------|-------|-------|--------|--------|--------|--------|--------|-------|-------|
| 1  | β-thujene                  | 971   | 968   | tr     | 0.2 ± 0.02 | -    | 0.1 ± 0.02 | 0.2 ± 0.02 | 0.1 ± 0.01 |
| 2  | propanoic acid, pentyl ester | 975   | 972   | -      | 0.3 ± 0.02 | -    | 0.1 ± 0.02 | 0.1 ± 0.01 |
| 3  | β-pinene                   | 982   | 978   | -      | 0.2 ± 0.02 | 0.2 ± 0.02 | -    | -      |
| 4  | β-myrcene                  | 987   | 987   | 57.7 ± 0.09 | 70.4 ± 0.02 | 71.4 ± 0.02 | 50.1 ± 0.02 | 31.8 ± 0.02 | 53.3 ± 0.04 |
| 5  | propanoic acid, 2-methyl-2-methylbutyl ester | 993   | 989   | -      | 0.3 ± 0.01 | 0.2 ± 0.02 | 3.1 ± 0.03 | tr    | 0.3 ± 0.02 |
| 6  | propanoic acid, 2-methyl-3-methylbutyl ester | 999   | 996   | -      | 0.9 ± 0.02 | -    | 0.2 ± 0.02 | -      | 1.8 ± 0.01 |
| 7  | limonene                   | 1033  | 1029  | 0.4 ± 0.02 | 0.9 ± 0.01 | 0.8 ± 0.02 | 0.6 ± 0.02 | 0.3 ± 0.02 | 0.6 ± 0.02 |
| 8  | cis-β-ocimene              | 104   | 1037  | 0.2 ± 0.02 | 0.2 ± 0.02 | -    | 0.1 ± 0.01 | tr    | -      | 0.2 ± 0.03 |
| 9  | methyl 6-methyl heptanoate | 1072  | 1068  | -      | -      | -    | -      | -      | 0.2 ± 0.02 |
| 10 | 2-nonanone                 | 1093  | 1091  | tr     | 0.4 ± 0.02 | 0.2 ± 0.01 | -    | 1.2 ± 0.02 | 0.6 ± 0.02 |
| 11 | linalool                   | 1094  | 1095  | 1.0 ± 0.02 | 0.8 ± 0.02 | 0.3 ± 0.02 | 0.7 ± 0.02 |
| 12 | butanoic acid, 3-methyl-3-methylbutyl ester | 1103  | 1100  | -      | 0.2 ± 0.02 | 0.8 ± 0.02 | -      | -      |
| 13 | perillen                   | 1105  | 1101  | -      | 0.4 ± 0.01 | -    | -      | -      |
| 14 | octanoic acid, methyl ester | 1125  | 1122  | -      | 0.1 ± 0.01 | -    | -      | -      |
| 15 | 3-octen-1-ol, acetate (Z)- | 1197  | 1194  | -      | -      | -    | -      | -      | 0.2 ± 0.01 |
| 16 | 2-undecanone               | 1283  | 1276  | 0.3 ± 0.02 | 0.4 ± 0.01 | 0.1 ± 0.01 | tr    | 0.8 ± 0.02 | 0.3 ± 0.02 | 0.7 ± 0.02 |
| 17 | 4-decenolic acid, methyl ester, Z-trans-geranic acid, methyl ester | 1295  | *     | -      | -      | -    | 0.8 ± 0.03 | 0.1 ± 0.02 | 0.9 ± 0.02 |
| 18 | Z-methyl geranate          | 1306  | 1302  | -      | 0.2 ± 0.01 | -    | -      | -      |
| 19 | α-cubebene                 | 1326  | 1324  | 0.6 ± 0.02 | 0.3 ± 0.02 | -    | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.02 | 0.3 ± 0.02 |
| 20 | α-gurjunene                | 1352  | 1350  | 0.3 ± 0.03 | -      | -    | -      | -      | 0.2 ± 0.01 |
| 21 | ylangene                   | 1375  | 1376  | -      | -      | -    | -      | 0.1 ± 0.01 | 0.2 ± 0.01 |
| 22 | copaene                    | 1391  | 1392  | -      | 0.6 ± 0.02 | 0.2 ± 0.02 | 0.1 ± 0.01 | 0.7 ± 0.02 |
| 23 | β-caryophyllene            | 1425  | 1422  | -      | 0.3 ± 0.02 | -    | -      | -      |
| 24 | selina-5,11-diene          | 1439  | 1440  | 16.2 ± 0.04 | 11.4 ± 0.04 | 1.7 ± 0.01 | 11.5 ± 0.03 | 8.7 ± 0.03 | 15.1 ± 0.04 | 8.7 ± 0.04 |
| 25 | aromadendrene              | 1446  | 1463  | 3.8 ± 0.02 | -      | -    | -      | -      |
| 26 | humulene                   | 1469  | 1470  | 19.0 ± 0.05 | 21.9 ± 0.02 | 10.8 ± 0.02 | 13.1 ± 0.02 | 30.7 ± 0.02 | 43.9 ± 0.02 | 10.9 ± 0.02 |
| 27 | α-eudesmene                | 1479  | 1481  | 2.3 ± 0.04 | 3.6 ± 0.04 | 1.2 ± 0.02 | 0.5 ± 0.01 | 0.2 ± 0.02 | 0.3 ± 0.02 | 2.0 ± 0.01 |
| 28 | alloaromadendrene          | 1482  | 1483  | -      | -      | -    | 0.7 ± 0.02 | 0.7 ± 0.02 | 0.9 ± 0.03 | -      |
| 29 | γ-muurolene                | 1489  | 1486  | -      | -      | 0.7 ± 0.02 | -    | 1.7 ± 0.03 | -      |
| 30 | δ-cadinene                 | 1508  | 1511  | 1.5 ± 0.02 | 0.4 ± 0.02 | tr    | 1.9 ± 0.02 | 2.7 ± 0.02 | 0.8 ± 0.02 |
| 31 | α-selinene                 | 1515  | 1512  | -      | 11.8 ± 0.02 | -    | -      | -      |
| No  | Component                     | LRI 1  | LRI 2  | STG(%) | HBK(%) | HLB(%) | CCH(%) | PCJ(%) | PC(%) | TP(%) |
|-----|-------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
| 34  | geranyl isobutyrate           | 1518   | 1514   | -      | -      | -      | -      | -      | -      | 1.7 ± 0.02 |
| 35  | 7-epi-α-selinene              | 1522   | *      | -      | -      | 0.2 ± 0.01 | -      | -      | -      | -     |
| 36  | selina-3,7(11)-diene          | 1530   | 1530   | -      | 4.2 ± 0.05 | -      | -      | -      | -      | -     |
| 37  | cadina-1(10), 4-diene         | 1540   | 1538   | 1.1 ± 0.02 | -      | -      | -      | -      | -      | -     |
| 38  | humulene epoxide 2            | 1610   | 1606   | -      | -      | -      | -      | 0.1 ± 0.01 | 0.3 ± 0.02 | -     |
| 39  | palmitic acid                 | 1977   | 1973   | -      | -      | -      | -      | -      | -      | 16.1 ± 0.02 |
| 40  | stearic acid                  | 2163   | 2158   | 0.8 ± 0.02 | -      | -      | -      | -      | -      | -     |

**SUM**

| 99.99 | 99.99 | 99.90 | 99.70 | 99.90 | 99.80 | 99.90 |

**Monoterpenes**

| 59.90 | 52.20 | 72.10 | 72.80 | 51.30 | 33.60 | 22.60 |

**Sesquiterpenes**

| 38.90 | 47.50 | 26.10 | 26.70 | 42.40 | 65.50 | -     |

**Other**

| 1.10  | 0.40  | 1.70  | 0.20  | 6.20  | 0.70  | 22.20 |

1 The components are reported according to their elution order on a polar column; 2 Linear Retention indices measured on apolar column; 3 Linear Retention indices from literature; * LRI not available; STG: Styrian Golding hop components; HBK: Hersbruker hop components; HLB: Hallertau Blanc hop components; CCH: “Cascade Cryo Hops®” hop components; PCJ: Pacific Jade hop components; PC: Pacifica hop components; TP: Topaz hop components; -: Not detected; tr: traces (mean value < 0.1).
What is noteworthy is the presence of palmitic acid (16.1%), which was only in TP hop.

3.2. H. lupulus Extract Chemical Composition

The chemical profile of the hop extracts was performed by GC/MS technique (Table 3). In all extracts, the content of sesquiterpenes was significantly higher than that of monoterpenes and ranged from 9.5% in TPe up to 35.4% in STGe. Among the monoterpenes, linalool was found in STGe, HBKe, CChE and PCe, while Z-methyl geranate (1.5%) and α-copaene (0.2%) were observed only in STGe and PCe, respectively. Several sesquiterpene compounds were identified among which β-caryophyllene, humulene, β-eudesmene and caryophyllene oxide were the most representative. In detail, β-caryophyllene was present in STGe (4.7%), HBKe (3.6%), CChE (3.6%), PCJe (3.7%), PCe (5.0%) and TPe (6.5%) while humulene was found in the same varieties (STGe: 12.0%, HBKe: 6.8%, CChE: 11.0%, PCJe: 14.1% and PCe: 17.8%), except for TPe. On the contrary, β-eudesmene was present only in STGe (7.4%) and in HLBe (35.5%). Caryophyllene oxide, present in average percentages lower than the compounds mentioned, was missing only in HLBe hop. Other minor sesquiterpenes were present only in one sample and missing in all others, particularly δ-cadinene (1.8% in STGe), seliná-3,7(11)-diene (1.7% in HBKe) and α-eudesmol (4.1% in HBKe). In all analyzed samples, the presence of lupulone was significant varying from 14.5% to 63.8%.

The SPME technique was also used to analyze the beer volatile composition. Fifteen compounds were identified (Table 4), among which eight belong to the fatty acid family. Only two terpene components such as β-myrcene and linalool were detected and, in particular, both compounds were found in IPA (2.0% and 0.8%, respectively) while only β-myrcene was present in the ACD sample (7.7%); on the contrary, they were missing in the LKO sample. The highest content of fatty acids was in the IPA sample (89.4%) followed by LKO (78.6%) and ACD (67.3%). In particular, hexyl octanoate (32.9%, 23.7%) and ethyl decanoate (23.4% and 15.3%) were the most abundant in LKO and in ACD, respectively. Hexadecanoic acid detected only in IPA (73.2%) represented the principal component.
### Table 3. Chemical composition (percentage mean value ± standard deviation) of hop methanol extracts.

| No. | COMPONENT | LRI 2 | LRI 3 | STGe(%) | HBKe(%) | HLBe(%) | CCHe(%) | PCJe(%) | PCe(%) | TPe(%) |
|-----|-----------|-------|-------|---------|---------|---------|---------|---------|--------|--------|
| 1   | 1,4-pentadiene | 555 | *    | -       | -       | -       | 0.2 ± 0.02 | -        | 5.7 ± 0.05 |
| 2   | 2-methyl-3-buten-2-ol | 603 | 601  | -       | -       | -       | 7.2 ± 0.02 | 0.5 ± 0.03 | 1.0 ± 0.04 |
| 3   | propane, 2,2-dimethoxy- | 650 | *    | -       | -       | -       | 1.1 ± 0.02 | -        | -      |
| 4   | 2-methylpropanoic acid, | 761 | 765  | -       | -       | -       | 1.6 ± 0.03 | 1.3 ± 0.02 | 2.1 ± 0.02 |
| 5   | 3-methylbutanoic acid | 831 | 839  | 3.0 ± 0.02 | 3.0 ± 0.07 | 4.6 ± 0.02 | 4.4 ± 0.03 | 1.5 ± 0.03 | 2.3 ± 0.04 | 2.4 ± 0.02 |
| 6   | 2-methylbutanoic acid | 840 | 848  | -       | -       | -       | 1.0 ± 0.02 | -        | -      |
| 7   | 1,6-octadiene, 2,7-dimethyl- | 870 | *    | -       | -       | 13.1 ± 0.02 | -        | 2.7 ± 0.03 | 4.8 ± 0.03 |
| 8   | 2(5 H)-furanone, 5,5-dimethyl- | 955 | 952  | 1.9 ± 0.02 | 0.6 ± 0.02 | -       | 2.2 ± 0.02 | 0.3 ± 0.02 | 0.9 ± 0.03 | 1.1 ± 0.02 |
| 9   | 2-methyl-2-pentenoic acid | 1000 | *    | -       | -       | -       | 8.2 ± 0.06 | 11.1 ± 0.08 | 7.2 ± 0.02 | 2.7 ± 0.04 | 8.0 ± 0.03 |
| 10  | linalool | 1098 | 1095 | 1.0 ± 0.03 | 0.7 ± 0.02 | -       | 0.5 ± 0.03 | -        | 1.0 ± 0.02 | -      |
| 11  | pyranone | 1125 | *    | 10.0 ± 0.05 | 5.0 ± 0.05 | 9.5 ± 0.03 | -        | -        | 3.3 ± 0.03 | -      |
| 12  | 2,3-dimethyl-2-hexanol | 1234 | *    | 11.4 ± 0.01 | -       | -       | -        | -        | 5.2 ± 0.02 | -      |
| 13  | 2-undecanone | 1281 | 1276 | -       | -       | -       | -        | -        | 3.2 ± 0.03 | -      |
| 14  | Z-methyl geranate | 1327 | 1323 | 1.5 ± 0.03 | -       | -       | -        | -        | -      |
| 15  | α-copaene | 1398 | 1392 | -       | -       | -       | -        | -        | 0.2 ± 0.02 | -      |
| 16  | β-caryophyllene | 1445 | 1440 | 4.7 ± 0.03 | 3.6 ± 0.02 | -       | 3.6 ± 0.02 | 3.7 ± 0.02 | 5.0 ± 0.07 | 6.5 ± 0.03 |
| 17  | humulene | 1478 | 1473 | 12.0 ± 0.03 | 6.8 ± 0.02 | -       | 11.0 ± 0.01 | 14.1 ± 0.02 | 17.8 ± 0.04 | -      |
| 18  | β-eudesmene | 1485 | 1481 | 7.4 ± 0.02 | -       | 35.5 ± 0.07 | -        | -        | -      |
| 19  | γ-muurolene | 1488 | 1486 | -       | -       | -       | -        | -        | 1.5 ± 0.02 | -      |
| 20  | δ-cadinene | 1509 | 1511 | 1.8 ± 0.02 | -       | -       | -        | -        | -      |
| 21  | geranyl isobutyrate | 1517 | 1514 | -       | -       | -       | 1.0 ± 0.04 | -        | 2.6 ± 0.04 | -      |
| 22  | selina-3,7(11)-diene | 1535 | 1530 | -       | 1.7 ± 0.02 | -       | -        | -        | -      |
| 23  | caryophyllene oxide | 1579 | 1583 | 6.0 ± 0.05 | 2.4 ± 0.01 | -       | 2.3 ± 0.02 | 1.7 ± 0.02 | 3.5 ± 0.04 | 0.8 ± 0.02 |
| 24  | humulene epoxide 2 | 1600 | 1606 | 18.4 ± 0.05 | 7.1 ± 0.02 | -       | 9.5 ± 0.02 | 5.7 ± 0.04 | 15.0 ± 0.07 | 1.4 ± 0.02 |
| 25  | α-eudesmol | 1612 | 1615 | -       | 4.1 ± 0.03 | -       | -        | -        | -      |
| 26  | neophytadiene | 1835 | 1840 | 3.5 ± 0.07 | 5.6 ± 0.02 | 9.6 ± 0.02 | -        | 2.0 ± 0.02 | 2.0 ± 0.02 | 2.2 ± 0.02 |
| 27  | lupulone | 2000 | *    | 14.5 ± 0.02 | 51.1 ± 0.03 | 16.0 ± 0.06 | 44.3 ± 0.05 | 63.8 ± 0.02 | 27.1 ± 0.02 | 55.7 ± 0.04 |
| 28  | SUM | - | - | 99.8 | 99.9 | 99.4 | 95.9 | 99.2 | 91.5 | 94.3 |
| 29  | Monoterpenes | 2.5 | 0.7 | - | 0.5 | - | - | - | 1.2 | - |
| 30  | Sesquiterpenes | 35.4 | 24.2 | 45.1 | 16.9 | 21.5 | 29.8 | 9.5 | - | - |
| 31  | Other | 61.9 | 75.0 | 54.3 | 78.5 | 78.4 | 60.5 | 84.8 | - | - |

1 The components are reported according to their elution order on a polar column; 2 Linear Retention indices measured on apolar column; 3 Linear Retention indices from literature; * LRI not available; STGe: Styrian Golding hop extract components; HBKe: Hersbruker hop extract components; HLBe: Hallertau Blanc hop extract components; CCHe:”Cascade Cryo Hops®” hop extract components; PCJe: Pacific Jade hop extract components; PCe: Pacifica hop extract components; TPe: Topaz hop extract components. -: Not detected; tr: traces (mean value <0.13.3). Vapor phase beer chemical composition.
Table 4. Chemical composition (percentage mean value ± standard deviation) of vapor phase beer samples.

| N° | COMPONENT                  | LRI 2 | LRI 3 | IPA(%) | LKO(%) | ACD(%) |
|----|----------------------------|-------|-------|--------|--------|--------|
| 1  | 3-methyl-1-butanol         | 738   | 740   | 2.5 ± 0.03 | 9.6 ± 0.03 | 8.2 ± 0.02 |
| 2  | 2-methyl-1-butanol         | 741   | 744   | 1.2 ± 0.02 | 3.5 ± 0.03 | 3.6 ± 0.03 |
| 3  | isovaleric acid            | 875   | 877   | 1.8 ± 0.02 | 3.0 ± 0.01 | 5.2 ± 0.02 |
| 4  | henthy acetate             | 980   | 981   | 1.1 ± 0.02 | 3.5 ± 0.03 | 2.4 ± 0.03 |
| 5  | β-myrcene                  | 987   | 989   | 2.0 ± 0.02 | -        | 7.7 ± 0.02 |
| 6  | linalool                   | 1094  | 1095  | 0.8 ± 0.03 | -        | -        |
| 7  | 2-phenyl ethanol           | 1100  | 1102  | 0.6 ± 0.02 | 4.5 ± 0.02 | 3.9 ± 0.01 |
| 8  | caprylic acid              | 1174  | 1178  | 2.4 ± 0.02 | 8.8 ± 0.02 | 13.3 ± 0.02 |
| 9  | hexyl octanoate            | 1196  | 1198  | 8.0 ± 0.02 | 32.9 ± 0.07| 23.7 ± 0.01 |
| 10 | phenethyl acetate          | 1230  | 1229  | 1.7 ± 0.02 | 0.8 ± 0.02 | 4.1 ± 0.01 |
| 11 | 2-methyl-2-pentenoic acid | 1254  | *     | 0.3 ± 0.02 | -        | -        |
| 12 | capric acid                | 1375  | 1373  | 0.7 ± 0.02 | 6.9 ± 0.04 | 10.5 ± 0.02 |
| 13 | ethyl decanoate            | 1398  | 1396  | 2.8 ± 0.02 | 23.4 ± 0.02| 15.3 ± 0.06 |
| 14 | ethyl dodecanoate          | 1596  | 1593  | 0.9 ± 0.03 | 3.1 ± 0.02 | 2.1 ± 0.02 |
| 15 | palmitic acid              | 1977  | 1973  | 73.2 ± 0.06 | -         | -         |
|    | SUM                        |       |       | 100.0  | 100.0   | 100.0   |
|    | Monoterpenes               | 2.8   | -     | 7.7    |         |         |
|    | Fatty Acids                | 89.4  | 78.6  | 67.3   |         |         |
|    | Other                      | 7.8   | 21.4  | 25.0   |         |         |

1 The components are reported according to their elution order on a polar column; 2 Linear Retention Indices measured on apolar column; 3 Linear Retention indices from literature; * LRI not available; IPA: Percentage mean values of “Ipagea” beer components (%); LKO: Percentage mean values of “Lokomotiv” beer components; ACD: Percentage mean values of “Accademica” beer components (%); -: Not detected; tr: traces (mean value < 0.1%).

3.3. Total Polyphenol and Flavonoid Content

The phenolic and flavonoid contents of the beer and hop extract samples are shown in Tables 5 and 6, respectively. Total polyphenols ranged from 27.4 to 49.8 mg GAE/g of dried hops and from 228.7 to 292.0 mg GAE/100 mL of beer. Total flavonoids varied between 6.3 and 22.0 mg QE/g of dried hops and between 3.1 and 5.8 mg QE/100 mL of beer. Among hop extracts, TPe showed the highest amount of polyphenols while PCJe had the highest amount of flavonoids. CChE hop was found to be the poorest in both polyphenols and flavonoids (Table 5). Among the beers, ACD was the richest in both classes of compounds. LKO beer exhibited a higher amount of polyphenols than IPA beer and both possessed similar flavonoid content (Table 6).

Table 5. Polyphenols and flavonoids of hop extract samples.

| Hops            | Total Polyphenols (mg GAE/g Dried Hops) | Total Flavonoids (mg QE/g Dried Hops) |
|-----------------|----------------------------------------|--------------------------------------|
| STGe            | 35.5 ± 0.5                             | 17.7 ± 0.0                           |
| HBKe            | 27.4 ± 0.4                             | 15.3 ± 0.1                           |
| HLBe            | 32.0 ± 0.0                             | 18.8 ± 0.0                           |
| CChE            | 23.0 ± 0.0                             | 6.3 ± 0.1                            |
| PCJe            | 37.0 ± 0.1                             | 22.0 ± 0.1                           |
| PCe             | 30.8 ± 0.1                             | 16.9 ± 0.1                           |
| TPe             | 49.8 ± 0.5                             | 20.6 ± 0.1                           |

HSTGe: “Styrian Golding” hop extract; HBKe: “Hersbrucker” hop extract; HLBe: “Hallertau Blanc” hop extract; CChE: “Cascade Cryo Hops®” hop extract; PCJe: “Pacifica Jade” hop extract; PCe: “Pacifica” hop extract; TPe: “Topaz” hop extract.
Table 6. Polyphenols and flavonoids of beer samples.

| Beer | Total Polyphenols (mg GAE/100 mL Beer) | Total Flavonoids (mg QE/100 mL Beer) |
|------|----------------------------------------|--------------------------------------|
| IPA  | 228.7 ± 1.8                            | 3.1 ± 0.1                            |
| LKO  | 248.3 ± 5.2                            | 2.8 ± 0.1                            |
| ACD  | 292.0 ± 0.0                            | 5.8 ± 0.1                            |

IPA: “Ipagea” beer; LKO: “Lokomotiv” beer; ACD: “Accademica” beer.

3.4. Antioxidant Activity

To determine antioxidant activity, DPPH and ABTS assays were carried out and the results were listed in Tables 7 and 8 for hop and beer samples, respectively. In DPPH assay, all samples revealed a good antioxidant activity and IC\textsubscript{50} values ranked from 0.027 for PCJe hop to 0.056 mg/mL for HBKe hop. TPe, PCe, CCHe, HLBe and STGe samples had quite similar IC\textsubscript{50} values (0.033, 0.047, 0.041, 0.039 and 0.039, respectively). The ABTS assay confirmed the antioxidant activity of all samples with IC\textsubscript{50} values slightly different from those obtained by the DPPH test. The range was from 0.015 for the HLBe hop sample to 0.134 mg/mL for CCHe hop sample. Low IC\textsubscript{50} values were also obtained for TPe and PCe samples (0.023 and 0.024, respectively). As reported in Table 7, the antioxidant activities by both assays were also expressed in TEAC.

Table 7. IC\textsubscript{50} and TEAC values of hop extract samples in DPPH and ABTS assays.

| STGe | HBKe | HLBe | CCHe | PCJe | PCe | TPe |
|------|------|------|------|------|-----|-----|
| DPPH | IC\textsubscript{50} * | 0.039 ± 0.007 | 0.05 ± 0.005 | 0.039 ± 0.005 | 0.041 ± 0.002 | 0.027 ± 0.008 | 0.047 ± 0.004 | 0.033 ± 0.001 |
|      | TEAC ** | 0.972 ± 0.200 | 0.66 ± 0.075 | 0.937 ± 0.074 | 0.891 ± 0.062 | 1.661 ± 0.013 | 0.771 ± 0.011 | 1.124 ± 0.097 |
| ABTS | IC\textsubscript{50} * | 0.041 ± 0.004 | 0.071 ± 0.014 | 0.015 ± 0.002 | 0.134 ± 0.009 | 0.037 ± 0.004 | 0.024 ± 0.005 | 0.023 ± 0.005 |
|      | TEAC ** | 0.380 ± 0.054 | 0.219 ± 0.023 | 1.036 ± 0.105 | 0.114 ± 0.003 | 0.413 ± 0.073 | 0.661 ± 0.095 | 0.684 ± 0.106 |

* mg/mL of hop samples; ** µmol of Trolox equivalent/ mg of dry hop samples. Values are expressed as means ± SD. p < 0.05. STGe: “Styrian Golding” hop extract; HBKe: “Hersbrucker” hop extract; HLBe: “Hallertau Blanc” hop extract; CCHe: “Cascade” hop extract; PCJe: “Pacifica Jade” hop extract; PCe: “Pacifica” hop extract; TPe: “Topaz” hop extract.

Table 8. IC\textsubscript{50} and TEAC values of beer samples in DPPH and ABTS assays.

| IPA | LKO | ACD |
|-----|-----|-----|
| DPPH | IC\textsubscript{50} * | 11.216 ± 0.424 | 5.174 ± 0.919 | 4.969 ± 0.671 |
|      | TEAC ** | 0.003 ± 0.00 | 0.007 ± 0.000 | 0.007 ± 0.001 |
| ABTS | IC\textsubscript{50} * | 0.244 ± 0.010 | 0.384 ± 0.009 | 0.198 ± 0.007 |
|      | TEAC ** | 0.048 ± 0.002 | 0.030 ± 0.001 | 0.058 ± 0.002 |

* v/v% of beer samples; ** µmol of Trolox equivalent/mL of beer samples. Values are expressed as means ± SD. p < 0.05. IPA: “Ipagea” beer; LKO: “Lokomotiv” beer; ACD: “Accademica” beer.

Beer samples were investigated to determine their antioxidant activities, and values were reported as IC\textsubscript{50} values, which were expressed in v/v% and TEAC values reporting the micromole (µmol) of Trolox equivalent/mL (Table 8). The ACD beer sample revealed the lowest IC\textsubscript{50} value, which correspond to the highest antioxidant activity, in the DPPH assay (4.969 v/v%) and in the ABTS assay (0.198 v/v%). TEAC values for ACD samples were 0.007 and 0.058 µmol of Trolox equivalent/mL for DPPH and ABTS, respectively.

4. Discussion

The volatile chemical profile of dried hops and beers was described by SPME sampling technique followed by GC/MS analyses. The obtained results from hop analyses by the SPME technique showed that monoterpene content exceeded that of the sesquiterpenes in all hop samples except for the PC sample where the ratio was reversed (Table 2). β-Myrcene was the predominant compound, and it is responsible for the pungent odor and aroma of hops [14]. In particular, the CCH sample was characterized by higher monoterpenic content.
(72.8%) of which β-myrcene was the most representative (71.4%). The high percentage of β-myrcene is due to the use of CRYO HOPS® technology (Yakima Chief Hops, LLC.-Yakima, WA, USA), an innovative hop production technique that allows the separation of lupulin from hop flower by cryogenic processing. The result of this technique is a product characterized by a more intense aromatic character with reduced astringency and vegetative material. In fact, the CCH hop sample was also found to be the poorest in both polyphenols and flavonoids (Table 5).

The other detected monoterpenes from hops (SPME collection) were present in markedly lower percentages ranging between 0.1% and 1.2%. Humulene and β-caryophyllene were sesquiterpene compounds more relevant in all hop varieties. Their ratio is used as a marker and it is characteristic of each variety [15]. Moreover, several esters were identified in HLB, CCH, PCJ, PC and TP samples while they were missing in STG and HBK hops. This class of compounds is reported to be responsible for characteristic odors with floral and fruity tones [14].

Regarding headspace beer compositions described in Table 4, we found a low content of monoterpenes (β-myrcene and linalool in IPA and only β-myrcene in ACD); on the contrary, secondary alcohols, such as 2-methyl-1-butanol and 3-methyl-1-butanol detected in the three beer samples, contributed mostly to the total amount of alcohols with percentage values ranging from 1.2% to 2.5% and from 2.5% to 9.6%, respectively. Several volatile compounds have been associated with specific flavors. For example, ethyl decanoate (from 2.8% to 23.4%) is associated with an apple aroma while linalool was found only in IPA (0.8%) with citrus and rose aromas [16].

Free fatty acids represent one group of beer flavor that affect beer taste and stability over time; medium-chain acids such as hexanoic, octanoic and decanoic acids are formed during fermentation. In our study, octanoic acid and hexadecenoic acids have been found to reach the highest value in the ACD sample (13.3% and 10.5%, respectively). In general, they have rancid flavor characteristics, and their formation is influenced by more factors, such as yeast strain, wort composition and the degree of aeration [17,18]. Different chemical families have an important impact on beer aroma properties [19]; for example, the esters ethyl hexanoate and ethyl octanoate (percentages in LKO > ACD > IPA) are responsible for sour apple aromas while 2-phenylethanol (percentages in LKO > ACD > IPA) and phenylethyl acetate (percentages in ACD > IPA > LKO) are responsible for rose aromas.

Among long-chain fatty acids, we found hexadecanoic acid (palmitic acid) only in IPA (73.3%). This result agrees with what is shown by the analysis of dried hop TP (Table 1) used for the production of this type of beer and the only variety where this fatty acid has been detected (16.1%).

On the other side, the investigation performed on hop methanol extracts (Table 3) showed an ubiquitous presence of sesquiterpenes in high percentages, including β-caryophyllene, humulene, β-eudesmene and caryophyllene oxide as the major exponents. These data are in agreement with those reported by Ligor et al. [20], who listed these compounds as the most important for hop aroma. Other minor compounds, such as 2-methylbutanoic acid, 3-methylbutanoic acid and 2-methylpropanoic acid, detected in variable percentages in the extracts play an important role for hop aroma because they produce a fruity smell [21].

Notably, the presence of β-acid lupulone, a bitter acid of hop cones, was found in all methanol extracts with percentage values ranging from 14.5% to 63.8%. This compound has been reported to have sedative, antiangiogenic, antiinflammatory properties and chemopreventive effects on human colon cancer and was also evaluated for in vivo antimicrobial activity to inhibit Clostridium perfringens [5,22–25]; 2-methyl-3-buten-2-ol, a degradation product of lupulone, was detected in CCH e (7.2%), PCJe (0.5%) and TP (1.0%), and it was also shown to be sedative in mice and antioxidant power [26,27]. Concentrations of bitter acids can vary within cultivars from year to year. The variation may depend on temperature differences or even on precipitation [28]. Furthermore, since the production of secondary metabolites is also under genetic control, the measured concentrations can be
useful for the partial identification of geographical origins. In fact, European and North American wild hops can be distinguished from each other thanks to the relative quantity of bitter acids [29].

In general, hop extracts have been reported as antioxidant agents and the strongest activity was noted for dried hop methanol extracts [30].

In our study, the antioxidant activity of hop methanol extracts was investigated by DPPH and ABTS assays, and the results were expressed by IC$_{50}$ and TEAC values. For all samples, low IC$_{50}$ values, which correspond to a high antioxidant potential, were obtained. Slight differences among them were observed: PCJe > TPe > STGe > HLBe > CCHe > PCe > HBKe in DPPH and HLBe > TPe > PCe > PCJe > STGe > HBKe > CCHe in ABTS assays, respectively. The PCJe sample, the hop with the highest antioxidant power (IC$_{50}$ of 0.027 mg/mL) among those tested against DPPH, showed the highest content of total flavonoids (22.0 mg QE/g dry hop) and a high amount of total polyphenols content (37.0 mg GAE/g dry hop). PCJe is also the sample with the highest relative percentage of lupulone (63.8%), for which its hydroxyl free radical scavenging activity was reported [31]. In ABTS assays, the HLBe hop was the most active (IC$_{50}$ of 0.015 mg/mL) and 32.0 mg GAE/g dry hop and 18.8 QE/g dry hop were found, revealing an interesting polyphenol and flavonoid content, respectively. Furthermore, the major activity of HLBe can also be attributed to its high content in sesquiterpenes, which are reported as powerful antioxidant agents [32]. TPe hop showed good antioxidant activity both in DPPH (IC$_{50}$ of 0.033 mg/mL) and (IC$_{50}$ of 0.023 mg/mL) in ABTS assay and the results for determination of polyphenols showed the highest amount (49.8 GAE/g dry hop and 20.6 QE/g dry hop). As reported by Keskin et al. [33], the methanol extract of the hop cones and leaves showed good radical scavenging activity (0.78 and 0.99 mg/mL, respectively) with higher polyphenolic content for the hop cone extract. Moreover, Arsene et al. [34] found a direct correlation of antioxidant activities and the total phenolic and flavonoid content in the ethanol extract of H. lupulus cones. Furthermore, Krofta et al. [35] showed that the high content of polyphenols was correlated with the antioxidant activity of hop samples, which in turn depend on the storage temperature and form of hops itself.

Among beer samples, all products showed relevant antioxidant activity. The ACD sample was the most active in DPPH and in ABTS assays (4.969 and 0.198 mg/mL, respectively), and it was the richest one in polyphenols and flavonoids (292.0 GAE/100 mL beer and 5.8 QE/100 mL beer, respectively). The H1 hop sample, which showed good antioxidant activity in both antiradical tests (0.039 and 0.041 mg/mL, respectively) and used in ACD sample production, was characterized by 35.5 mg GAE/g dry hops of total polyphenols and 17.7 mg QE/g dry hops total flavonoids. STG hop was investigated by Guillaume et al. [36], and its antioxidant activity was determined as the inhibition time of linoleic acid-induced oxidation. The results showed that the higher the polyphenol or flavonoid content, the longer the inhibition time, and STG hop revealed significant antioxidant activity.

Beer antioxidant activity is a result of the presence of antioxidant compounds such as polyphenols and flavonoids and a correlation between total polyphenols, total phenolic acid content and the antioxidant activity of beers measured by FRAP assay was found [37]. Zhao et al. [38] studied 40 lager beers by different antioxidant assays and determined a positive correlation between the phenolic compounds and the antioxidant activities underlining the influence of oxidative reactions in flavor stability and in beer shelf life.

5. Conclusions

The present study aimed to identify, by SPME-GC/MS techniques, the VOCs from seven different hop cultivars and three beers as final products of brewing process to characterize their volatile profiles. Esters, alcohols, monoterpene and sesquiterpene compounds were detected with marked differences between investigated dried hop cones. The volatile content in the three craft beers was similar even if with different relative percentages for the same constituents and, among them, the IPA sample had the richest in components.
Furthermore, a chemical investigation was also carried out on hop methanol extracts used to measure polyphenolic and flavonoidic contents and to test the antioxidant power. Both hops and beers confirmed interesting antioxidant properties showing a positive correlation with polyphenolic and flavonoidic contents and acting as possible sources of bioactive compounds useful for exerting beneficial effects on human health following their consumption.

Author Contributions: Conceptualization, M.I. (Matteo Iannone) and S.G.; methodology, S.V., V.L.M., A.M. and S.G.; writing—original draft preparation, M.I. (Matteo Iannone), E.O., S.V. and S.G.; writing—review and editing, M.I. (Matteo Iannone), E.O., S.V. and S.G.; data curation, E.O., S.V., V.L.M. and S.G.; funding, M.I. (Marcello Iriti), A.T. and S.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All generated data are included in this article.

Acknowledgments: The authors are thankful to Circolo ARCI La Staffetta, Italy, for providing hops material (Styrian Goldings; Hersbrucker; Hallertau Blanc; Cascade Cry-o-Hop; Pacific Jade; Pacifica; Topaz) and beers (Ipagea; Lokomotiv; Accademia).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Magalhães, P.J.; Carvalho, D.O.; Cruz, J.M.; Guido, L.F.; Barros, A.A. Fundamentals and health benefits of xanthohumol, a natural product derived from hops and beer. Nat. Prod. Commun. 2009, 4, 591–610. [CrossRef] [PubMed]
2. Zanoli, P.; Rivasi, M.; Zavatti, M.; Brusiani, F.; Baraldi, M. New insight in the neuropharmacological activity of Humulus lupulus L. J. Ethnopharmacol. 2005, 102, 102–106. [CrossRef]
3. Mouratidis, P.X.E.; Colston, K.W.; Tucknott, M.L.; Tyrrell, E.; Pirianov, G. An Investigation into the Anticancer Effects and Antioxidative, Antimicrobial, and Anticarcinogenic Effects. Nutrients 2021, 13, 517. [CrossRef] [PubMed]
4. Wei, S.; Sun, T.; Du, J.; Zhang, B.; Xiang, D.; Li, W. Xanthohumol, a prenylated flavonoid from Hops, exerts anticancer effects against gastric cancer in vitro. Oncol. Rep. 2018, 40, 3213–3222. [CrossRef] [PubMed]
5. Siegel, L.; Miterrnique-Grosse, A.; Griffon, C.; Klein-Soyer, C.; Lobstein, A.; Raul, F.; Stephan, D. Antiangiogenic Properties of Lupulone, a Bitter Acid of Hop Cones. Anticancer Res. 2008, 28, 289–294. [PubMed]
6. Hrnčič, M.K.; Španinger, E.; Košir, I.J.; Knez, Z.; Bren, U. Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Antiinfectious Effects. Nutrients 2019, 11, 257. [CrossRef]
7. Gerhauser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauf, J.; Neumann, I.; Scherf, H.R.; Frank, N.; Bartsch, H.; et al. Cancer Chemopreventive Activity of Xanthohumol, a Natural Product Derived from Hop. Mol. Cancer Ther. 2002, 1, 959–969.
8. Roehrer, S.; Stork, V.; Ludwig, C.; Minecva, M.; Behr, J. Analyzing bioactive effects of the minor hop compound xanthohumol C on human breast cancer cells using quantitative proteomics. PLoS ONE 2019, 14, e0213469. [CrossRef]
9. Kaiser, T.C. Commercial Aspects of Hops; The 1st Hops Academy: Nürnberg, Germany, 2011.
10. Vitalini, S.; Iriti, M.; Vinciguerra, V.; Garzoli, S. A Comparative Study of the Chemical Composition by SPME-GC/MS and Antiradical Activity of Less Common Citrus Species. Molecules 2021, 26, 5378. [CrossRef]
11. Ovidi, E.; Laghezza Masci, V.; Zambelli, M.; Tiezzi, A.; Vitalini, S.; Garzoli, S. Laurus nobilis, Salvia sclarea and Salvia officinalis Essential Oils and Hydrolates: Evaluation of Liquid and Vapor Phase Chemical Composition and Biological Activities. Plants 2021, 10, 707. [CrossRef]
12. Xiao, F.; Xu, T.; Lu, B.; Liu, R. Guidelines for antioxidant assays for food components. Food Front. 2020, 1, 60–69. [CrossRef]
13. Garzoli, S.; Laghezza Masci, V.; Franceschi, S.; Tiezzi, A.; Giacomello, P.; Ovidi, E. Headspace/GC–MS Analysis and Investigation of Antibacterial, Antioxidant and Cytotoxic Activity of Essential Oils and Hydrolates from Rosmarinus officinalis L. and Lavandula angustifolia Miller. Foods 2021, 10, 1768. [CrossRef] [PubMed]
14. Van Opstaele, F.; De Causmaecker, B.; Aerts, G.; De Cooman, L. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography-mass spectrometry/olfactometry. J. Agric. Food Chem. 2012, 60, 12270–12281. [CrossRef] [PubMed]
15. Kralj, D.; Zupanec, J.; Vasilij, D.; Kralj, S.; Pšenčnik, J. Variability of Essential Oils of Hops, Humulus lupulus L. J. Inst. Brew. 1991, 97, 197–206. [CrossRef]
16. Liu, S.Q.; Quek, A.Y.H. Evaluation of beer fermentation with a novel yeast Williopsis saturnus. Food Technol. Biotechnol. 2016, 54, 403. [CrossRef]
17. Clapperton, J.F. Caprylic flavour as a feature of beer flavour. J. Inst. Brew. 1978, 84, 90–92. [CrossRef]
18. Clarke, B.J.; Davine, D.F.; Hawthorne, D.B.; Kavanagh, T.E.; Moulder, P.J. Factors affecting the formation of medium chain fatty acids during fermentation. Tech. Q. Master Brew. Assoc. Am. 1981, 18, 188–194.
19. Meilgaard, M.C.; Dalgliesh, C.E.; Clapperton, J.F. Beer flavour terminology. J. Inst. Brew. 1979, 85, 38–42. [CrossRef]
20. Lermusieau, G.; Liégeois, G.; Liégeois, C.; Collin, S. Reducing power of hop cultivars and beer ageing. Food Chem. 2001, 72, 413–418. [CrossRef]
21. Brendel, S.; Hofmann, T.; Granovgl, M. Hop-induced formation of ethyl esters in dry-hopped beer. Food Prod. Proc. Nutr. 2020, 2, 1–7. [CrossRef]
22. Sikorski, H.; Rusiecki, W. The sedative action of various constituents of hops. Bull. Intern. Acad. Polon. 1938, 1938, 76–83.
23. Hall, A.J.; Babish, J.G.; Darland, G.K.; Carroll, B.; Konda, V.R.J. Safety, efficacy and anti-inflammatory activity of rho iso-alpha-acids in the chicken intestinal tract jejunum and caecum. J. Antimicrob Chemother. 2008, 62, 1534–1547. [CrossRef]
24. De Keukeleire, J.; Janssens, I.; Heyerick, A.; Ghekiere, G.; Cambie, J.; Roldán-Ruiz, I.; Bockstaele, E.V.; Keukeleire, D.D. Relevance of organic farming and effect of climatological conditions on the formation of α-acids, β-acids, desmethylxanthohumol, and xanthohumol in hop (Humulus lupulus L.). J. Agric. Food Chem. 2007, 55, 61–66. [CrossRef]
25. Tagashira, M.; Watanabe, M.; Uemitsu, N. Antioxidative activity of hop bitter acids and their analogues. J. Agric Food Chem. 2007, 55, 65, 116–121. [CrossRef]
26. Wohlfart, R.; Hänsel, R.; Schmidt, H. Nachweis sedativ-hypnotischer Wirkstoffe im Hopfen. Phytochemistry 1982, 21, 934–941. [CrossRef]
27. Siragusa, G.R.; Haas, G.J.; Matthews, P.D.; Smith, R.J.; Buhr, R.J.; Dale, N.M.; Wise, M.G. Antimicrobial activity of lupulone against Clostridium perfringens in the chicken intestinal tract jejunum and caecum. J. Sci. Food Agric. 2007, 87, 1534–1547. [CrossRef]
28. Zhao, H.; Li, H.; Sun, G.; Yang, B.; Zhao, M. Assessment of endogenous antioxidative compounds and antioxidant activities of lager beers. J. Sci. Food Agric. 2013, 93, 910–917. [CrossRef] [PubMed]