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Relationship among Volatile Organic Compounds and Sensory Properties in Craft Beer

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Abstract: Hops flowers are used to impart highly desirable hoppy aromas in beer. The emergence of craft brewing caused an increase in the popularity of intense hoppy beer determining a breeding trend for new hop flavour varieties that differ in terms of oil contents and compounds. The aim of this work is to examine the relationship between volatile organic compounds (VOCs) and sensory properties in an Italian craft beer brewed with 2 selected Italian wild hop varieties and a commercial one (Cascade) grown in 2 sites with different environmental condition. Since the beer aroma is represented by hop flowers and so they increase incise in the finished product. In this study, 6 beer samples produced by an Italian microbrewery using hop plants were collected and analysed for Volatile Organic Compounds (VOCs) profiles using a PTR-TOF-MS and a sensory evaluation (panel and consumer tests). Multivariate statistical analyses (PCA and CANOCO) showed as "Cascade commercial" sample marks with the highest intensity of taste in comparison to other samples. Results showed low interest for the aromas the hops imparted to the beers produced in relationships to the commercial variety grown and bought. In addition, the grown commercial cascade resulted to be interesting, producing a modified aroma profile when compared to its commercial counterpart. Finally, this study showed an initial contribution to screen other wild genotypes to identify new hops for direct use or breeding with new characteristics that can be used for the production of beer with a modified aroma.

Keywords: wild hops, flavour, volatile organic compounds, panel test, consumer acceptability, CANOCO, PCA

1. Introduction

Hops (Humulus lupulus L.) flowers are used to impart a highly desirable hoppy aromas in beer. The aroma profiles, determining pleasant characteristics in the final products, are defined by the essential oil composition and their relative amounts present in the lupulin glands of the non-pollinated mature female inflorescence. The composition of essential oil compound in these inflorescence can be very diverse. Van Opstaele et al. [1] have identified more than 450 volatiles and suggested that hop oil may encompasses over 1000 different volatile compounds. Hops represent the principal ingredient producing the aroma, taste, foam stability, antimicrobial effects of beer, as well as several other applications in the pharmaceutical and food industries [2, 3].

The craft beer production has started in United States in around 1970s and a strong renaissance has been noticed recently [4]. Starting from 1990s, Italy has undergone through a steep growth of the craft beer sector as well [5, 6] which nowadays has expanded over 700 active craft brewery around the peninsula [7]. Cultivated hops has gradually decreased in Italy till few years ago. Indeed, a several small breweries are renewing the interest for local raw materials and biodiversity [8].

The emergence of craft brewing caused an increase of the popularity of intense hoppy beer styles determining a breeding trend for new hop flavour varieties that differ in terms of oil contents and compounds [9]. Along with this trend due to the strong interest for new hops, besides the development of cultivars and varieties from ecotypes with particular aroma qualities and satisfactory yield, Italian wild hops are screened nowadays for their volatile organic compounds (VOCs), genetic and morphological properties in comparison to commercial cultivars [29]. Indeed, hops plant produce different cones quality in relationship to their growth area.

Therefore, the main objective of this study was to investigate the relationship of VOCs and sensory properties in an Italian craft beer brewed using 2 selected autochthone hop genotypes (coming from two Italian regions) and a commercial

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one (Cascade) grown in 2 different environmental condition.

2. Materials and methods

2.1 Hops production and beer preparation

The first step was to find wild hops genotypes that could work since several wild hops showing undesirable characteristics are present on the territory. For the hop rhizomes retrieval, the Italian Hop Association (www.luppolo.org) selected some among the best genotypes found by its partners around the peninsula. Initially 29 varietal accessions were screened for harvesting hop cone from their original places and comparing them with commercially grown hops bought from Mr Malt online dealer (www.mr-malt.it). The cones harvested were dried, packaged and stored following the same shared protocols.

To carry out the real study phase, after the above described preliminary rhizomes screening, commercial rhizomes were bought from www.eickelmann.de. Further, the wild rhizomes were collected following the same protocol and placed into 15 cm diameter pots and transferred to the University of Florence (DAGRI Department). The plants were grown under controlled condition within a climatic chamber at 25°C, 12 hours of light per day, with a photosynthetically active average radiation of 300 μmol m-2 s-1 at the bud height.

Hops plant were then transplanted and cultivated in two different locations (i.e. Sant'Andrea a Morgiano, Florence, Italy and Travalle, Calenzano, Florence, Italy; Figure 1.) having substantially distinct soil and climatic characteristics: Both sites (Figure 1.) were adopted the same hop yard design, trellis system, plant density (row and plant spacing), site preparation, irrigation systems, planting and farming management.

Figure 1. Hop trellis planted in the experimental field in Sant'Andrea location

The ideal harvest period, and protocols for preparing and storing the cones were chosen on the base on the info retrieved from the American home brewer Association [10].

The beer utilized for the test was chosen among those already produced by the Microbrewery "I Due Mastri" identifying the most neutral one to enhance the characteristics and differences of the hops under test. Indeed, it was chosen a simple Pale Ale with 4% ABV, made with water, Italian barley, Tuscan sorghum, hops and yeast. However, its original hop bill wasn't added to the wort that instead was just dry hopped with 5g/L of single hop cones per thesis for a period of 5 days. The yeast used was the Fermentis SafAle™ US-05 fermented at 19°C in order to obtain a neutral profile. Only the two most promising hops were used. Afterwards, the beers obtained have been bottled (0.5 L bottles), carbonated through secondary fermentation (15 days) with the sugar needed to obtain 2.3 CO_2 vol. and finally stored at 4°C. At the end were obtained 10 liters bottled finished beer for each of the following thesis:

Reference beer

"Cascade", cultivated in Sant'Andrea;

"Cascade", cultivated in Travalle;

"Cascade", commercial;

Wild hops harvested from Rignano sull'Arno (Florence, Italy) namely "Troghi" and cultivated in Sant'Andrea site;

Wild hops harvested from Cherasco (Cuneo, Italy) namely "Piemonte 9" and cultivated in Sant'Andrea site;

2.2 VOC detection from different beer samples

The volatile compounds were analyzed with the help of a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) . The samples were analyzed using the instrumental parameters as suggested by Ritcher et al. [11]. In particular, the drift voltage and temperature were set respectively at 520 V and 110°C, which resulting in an E/N value of ~ 130 Townsend (Td, 1 Td = 10−17 cm2/V s). In this way, we have avoided the formation of ethanol clusters which might affect the final quantification of volatiles. Each sample was prepared on the basis of the following protocol: 10 ml of beer (T 20°C) were introduced in apposite glass jar (750 ml), which were fluxed with clean air (Zero air generator, Peak scientific) for 60 seconds and then sealed hermetically and incubated for 60 seconds at 20°C inside an incubator. VOCs sampling was made for 120 second, then, at the end of each sample, the instrument was cleaned using clean air fluxed for five minutes, and subsequently, another sample was analyzed. Each sample was analyzed in triplicate. As suggested by Aprea et al. [12], each sample was diluted applied the air obtained from the zero-air generator (1:2) to minimize measurement problems due to high ethanol concentrations.

2.3 Data processing

Data was extracted and pre-processed using a method described by Cappellin et al. [13]. The raw spectral data (expressed in number of counts per second, cps) were converted to ppbv as described by Lindinger et al. [14]. Subsequently, after subtracted the background (empty jar) were eliminated the ions not related to the sample (O2+, NO+ and ethanol and/or water clusters) and all signals with an amount below to 0.50 ppbv. At the end a total of 75 compounds (Table 3) were tentatively identified by comparing them to the literature Compounds were identified by comparison of their MS spectra (MS) and their retention times (S) with those of pure standards when available. To gain an overview of the different VOCs within the different types of beers a principal component analysis (PCA) was performed on the full data set.

2.4 Sensory evaluation

Two different panels (Panel test and Consumer test) of assessors were recruited for the study. The first panel were constituted of expert (with high technical expertise on beer, brewing and sensory evaluation), while the other one was based on the consumers (with beer expertise). All the evaluation was conducted inside a classroom located inside the University of Florence (DAGRI - Department) while the samples preparation took place in the adjacent classroom, to guarantee: 1) the standardization of the environmental conditions; 2) the reduction of psychological distractions and interactions among the subjects to perform the test; 3) the achievement of an adequate level of comfort.

2.4.1 Panel test

A panel of 8 trained judges was selected for beer sensory characterization (taste and aroma). The panel members had a minimum of 5 and a maximum of 20 years of experience in beer sensory evaluation. Commercial beers together with a craft beer were also used to standardize our panel's attributes definitions according to literature. The sensory descriptors and rules were chosen based on the ASBC methods [15]. The scoring of each sensory attribute selected was done on a five-point intensity scale, where 1 point means 'recognizable' and 5 points means 'strong' [16]. While aroma intensity, aroma persistence, tropical, citrus, herbal, resinous, floral, spicy, soapy, papery, cereal and honey as attributes were selected for the "aroma evaluation", the taste intensity, bitterness, bitterness persistence, sourness, sweetness and astringency were selected for the "taste evaluation". Subsequently, craft beers samples were presented in 80 ml glass covered with a glass top and containing 20 ml beer per glass. Bottles of each samples were stored in fridge until prepared. Immediately before tasting they were poured and served at 10˚C. Samples were coded with a randomly selected three-digit number. All the samples were evaluated within individual booths and replicated twice. Water was supplied to rinse the palate between samples.

2.4.2 Consumer acceptability

Consumer test represent methods of investigation, used in sensory sciences, of the degree of appreciation perceived by consumers, referring to one or more products. The method chosen for the study uses a 9-point scale, which measures the acceptability of the products, so the test aims to identify if they are present significant and real differences perceived by consumers with respect to the samples administered. In total, 80 judges were recruited among untrained regular beer consumers allowing to obtain a fairly reliable result [17]. The judges were represented by 50 women and 50 men, aged between 22 and 70 years, selected within research staff and students at Florence University. The judges' selection was based on their familiarity and consumption of beer. This was done in order to define a group of potential consumers representative of the national demographic profile. Besides the beers produced with the above-mentioned hops, a "Reference" was made and produced without any hops addition. Each beer was presented using 80 ml transparent plastic cups poured with 75 ml of beer [17]. The order of the presentation of the samples was randomized and balanced to avoid errors of central tendency, presentation, expectation, etc. In addition, each administration was accompanied by a random three-digit number [17]. The beers were served from the cold room at a controlled temperature of 8-10°C. A total of 80 judges have participated to evaluate samples for preference liking based on a 9-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely) [18]. The consumer acceptance was calculated as the percentage of consumer respondents who liked the sample, with scores >5; a score of six is considered a commercial quality limit [19].

2.5 Statistical analyses

In order to determine the relative correspondence between the consumer test 12 aroma variables and the 75 VOCs variables, considering the 6 different kind of beers (observations), a Canonical Correspondence Analysis (CANOCO) was carried out [20, 21]. Being an ordination technique, CANOCO was applied to observe in a same multidimensional space for the contributions of both consumer test aroma variables and VOCs matrices. The ordination axes are linear combinations of the aroma variables. CANOCO was performed using the free software PAST 2.17c [22] (http://folk.uio.no/ohammer/past/). In addition, an ANOVA was performed using the free software PAST 2.17c [22] (http://folk.uio.no/ohammer/past/).

3. Results and discussion

3.1 VOCs evaluation

The composition of VOCs varied greatly with a minimum of thirty-one compounds observed in the reference beer and maximum seventy-three in the beer obtained using the Cascade hops cultivated in "Travalle" place (Table 2). Thus, it is reasonable to assume that about two/thirds of the total VOCs identify, are linked to the hops action. Indeed, as reported elsewhere [23, 24], the addition of hops during the dry-hopping phase causes a substantial increase in the beer aroma.

Table 2. Consumer acceptability: average scores (with standard deviation) and percentage of responses (level > 5 of the nine-point hedonic scale). Different capital letter within a column indicates differences by the LSD test at the 99.0% confidence level (p = 0.01). Different letters represent statistically significant differences according to the One-Way ANOVA and Student's t-test for values of p < 0.05

Beer samples	Average scores	Response percentage % (level > 5 of the nine-point hedonic scale)
Cascade_commercial	7.30±1.40a	81
Cascade from "Sant'andrea"	5.80±0.70ab	52
Cascade_from "Travalle"	6.90±1.10a	75
Wild hops (Piemonte 9)	5.20±0.90b	31
Wild hops (Troghi)	4.50±1.50b	23
Reference	4.10±2.00b	28

For this reason, we evaluated the VOC fingerprints of beer obtained from different hops either commercial orwild. By analysing all the beers (Table 1.), a maximum of 73 volatile compounds were detected within a mass range between of m/z 20 and 250, which mainly include compounds such as terpenes, terpenoids, alcohols, esters, sulphur and acids. The number of total peaks and their intensities were different among the studied beers (Table 3.). In particular, were observed a number of compounds between 67 (wild hops) and 73 (commercial hops). Likewise, the "Reference Beer", showed both the lowest number of compounds detected and the total VOC emission intensity highlighting a rather simple aromatic profile (Table 3.). On the contrary, the beer obtained with a cascade hops (either commercial than when cultivated in "Travalle" site) showed a more complex aromatic profile compared to the beers obtained using a wild hops (Table 3.).

Table 1. Taste evaluation: average scores (with standard deviation) of taste profile of beers using a five-point intensity scale

Beer Samples	Taste intensity	Bitter	Bitter persistence	Sourness	Sweetness	Astringency
Cascade_commercial	3.50	3.20	3.20	1.41	1.35	1.39
Cascade from "Sant'andrea"	2.41	2.23	2.16	1.27	1.41	1.33
Cascade_from "Travalle"	3.10	3.00	2.52	1.37	1.27	1.50
Wild hops (Piemonte 9)	2.43	2.50	2.28	1.17	1.23	1.21
Wild hops (Troghi)	2.61	2.44	2.38	1.25	1.31	1.33
Reference	2.25	2.10	1.50	1.31	1.41	1.25

Table 3. Compounds identified through PTR-Analysis. a Protonated measured m/z; b Putative identifications; c Compound's Chemical formula (H+ added by protonation); d Compounds related to their bibliography: *Flavour - active volatile compounds in beer: production, regulation and control; # Scale - up of Dry Hopping Trials: Importance of Scale for Aroma and Taste Perceptions; ^Rapid analysis of selected beer volatiles by atmospheric pressure chemical ionisation-mass spectrometry; & Assessment of the aroma profiles of low-alcohol beers using HS-SPME–GC-MS; X Recognition of beer brand based on multivariate analysis of volatile fingerprint; q Onion-like off-flavour in beer: Isolation and identification of the culprits; § Optimization of the HS-SPME–GC–IT/MS method using a central composite design for volatile carbonyl compounds determination in beers. At the bottom: total VOCs amount and total peaks detected are shown

M/Za	Putative identificationb	Chemical Formulac	Reference beer		Cascade "S. Andrea"		Cascade "Travalle"		Cascade "Commercial"		Wild hops "Piemonte 9"		Wild hops "Toscana (Troghi)"		Referenced
			Average	Dev.St.	Average	Dev.St.	Average	Dev.St.	Average	Dev.St.	Average	Dev.St.	Average	Dev.St.	
27.022	Acetylene/Fragments	$C_2H_3^+$	4388.92	696.17	5027.05	941	9507.03	1192.48	9454.19	1159.46	5986.76	1180.7	7638.1	1295.24	
31.020	Formaldehyde	CH_3O^+	111.95	12.9	182.38	39.26	475.41	25.69	1034.88	50.5	223.39	8.14	287.77	14.48	
39.020	Isoprene fragment	$C_3H_3^+$	286.7	10.48	214.6	16.07	328.9	16.76	387	24.33	256.85	10.2	320.66	22.87	
41.037	Fragment (alcohol, ester, acetate)	$C_3H_5^+$	4691.59	386.06	4445.4	207.96	5514.31	380.35	6141.05	429.34	4365.27	189.81	5348.02	307.65	
43.018	Fragment (ester)	$C_2H_3O^+$	3492	1937.99	2464	183.13	4239	884.46	3649.5	872.61	2267.08	248.36	3378.39	896.8	
43.054	Fragment (alchol)	$C_3H_7^+$	281.97	320.56	102.72	5.95	132.91	5.71	154.14	7.8	115.9	9.85	131.48	11.26	
45.033	Acetaldehyde	$C_2H_5O^+$	410.18	20.66	404.41	30.96	762.19	49.28	936.6	69.64	417.71	27.13	551.34	32.04	*
47.047	Ethanol	$C_2H_7O^+$	86.66	38.66	51.54	2.51	228.09	74.81	207.25	43.37	55.09	4.14	121.92	12.45	&
49.011	Methanethiol	CH_5S^+	4.75	1.7	4.04	0.49	4.75	0.56	5.91	0.85	4.76	0.68	4.12	0.9	q
51.022	Methanethiol isotope	CH_5S^+	1.74	0.72	1.18	0.59	2.8	0.92	2.48	0.71	1.36	0.24	2.5	0.74	
53.038	Fragment	$C_4H_5^+$	9.76	2.69	13.81	3.3	50.94	4.02	24.28	3.13	13.88	1.43	15.93	3.2	
55.05	Fragment	$C_4H_5^+$	266.4	33.02	269.23	18.5	431.55	47.71	421.74	29.01	266.79	14.13	339.35	22.17	
57.033	Fragment	$C_3H_5O^+$	109.48	29.71	134.21	8.58	138.26	16.49	144.93	13.5	124.71	4.68	116.56	6.03	
57.069	Fragment	$C_4H_9^+$	1585.71	215.4	1666.01	73.06	1844.75	186.5	1833.59	165.11	1481.04	101.24	1943.9	111.32	
51.022	Methanethiol isotope	CH_5S^+	1.74	0.72	1.18	0.59	2.8	0.92	2.48	0.71	1.36	0.24	2.5	0.74	
53.038	Fragment	$C_4H_5^+$	9.76	2.69	13.81	3.3	50.94	4.02	24.28	3.13	13.88	1.43	15.93	3.2	
55.05	Fragment	$C_4H_5^+$	266.4	33.02	269.23	18.5	431.55	47.71	421.74	29.01	266.79	14.13	339.35	22.17	
57.033	Fragment	$C_3H_5O^+$	109.48	29.71	134.21	8.58	138.26	16.49	144.93	13.5	124.71	4.68	116.56	6.03	
57.069	Fragment	$C_4H_9^+$	1585.71	215.4	1666.01	73.06	1844.75	186.5	1833.59	165.11	1481.04	101.24	1943.9	111.32	
69.033	Furan	$C_4H_5O^+$	6.38	2.39	8.95	1.84	17.99	3.31	21.3	3.3	12.7	1.97	10.85	1.21	
69.069	Isoprene	$C_5H_9^+$	24.91	5.54	35.22	2.93	99.72	12.93	126.88	20.07	51.15	5.76	58.2	6.3	
71.048	(E)-2-butenal	$C_4H_7O^+$	96.99	35.71	123.6	8.47	186.86	26.33	176.75	25.39	109.96	5.31	104.95	2.82	§
71.083	alchol fragment (i.e Amyl alcohol)	$C_5H_{11}^+$	1353.15	309.6	1067.78	78.51	1095.76	150.88	1484.4	266.94	1009.62	96.45	1419.13	26.98	^
73.022	n.i.	$C_3H_5O_2^+$	0.99	0.27	3.41	1.94	1.68	0.82	4.23	1.46	1.4	0.52	4.02	0.69	
73.065	Butanal/2-Butanone	$C_4H_9O^+$	16.89	1.48	23.69	2.94	44.06	7.23	48.58	10.25	22.86	5.59	30.84	2.14	§
75.043	isobutyl alcohol/ Isobutanol	$C_4H_{11}O^+$	174.9	44.13	277.25	29.28	324.62	36.93	405.44	52.33	208.68	15.71	274.64	13.87	*
77.039	1-Propanethiol	$C_3H_9S^+$	28.34	6.39	21.24	1.95	60.07	4.16	55.7	3.55	24.21	2.81	37.28	1.71	
79.054	2-Mercaptoethanol	$C_6H_7^+$	14.57	5.13	25.97	2.84	90.36	11.83	62.94	4.57	31	4.17	40.87	8.98	
81.069	Fragment (C6 and Terpene Compounds)	$C_6H_9^+$	1.47	1.04	73.39	8.44	462.76	86.66	110.11	6.01	53.59	6.83	41.48	2.85	
83.050	n.i.	$C_5H_6O^+$	2.19	1.62	5.64	1.61	11.07	2.32	11.15	1	7.46	1.38	7.07	0.41	
83.084	n.i.	$C_6H_{11}^+$	0	0	2.62	1.6	2.07	0.72	2.23	0.56	0	0	0	0	
85.064	n.i.	$C_5H_9O^+$	2.62	0.98	6.98	0.91	17.67	4.59	17.07	1.63	8.73	1.88	7.55	1.25	
85.101	n.i.	$C_6H_{13}^+$	0.59	0.06	0	0	1.29	0.56	1.32	1.21	0	0	0	0	

87.044	2,3 - Butanedione (diacetyl)	$C_4H_7O_2^+$	6.62	2.13	6.33	1.22	12.15	2.96	12.82	1.77	8.72	2.61	10.01	1.27	-
87.080	Prenol/Pentanal/3-Methyl-1-butanal	$C_5H_{11}O^+$	17.34	3.34	22.36	4.71	44.94	23.46	67.69	8.09	19.82	2.8	28.5	2.48	§
89.060	Ethyl acetate	$C_4H_9O_2^+$	1119.88	58.19	2183.88	450.12	1982.31	396.54	2071.84	304.54	1533.71	196.67	2033.8	61.97	*
91.024	S-Methylthioacetate	$C_3H_7OS^+$	3.85	0.47	5.53	1.05	8.42	1.88	9.27	1.43	3.98	0.79	5.09	0.46	
93.069	Terpenes fragments (β - Caryophyllene)	$C_7H_9^+$	3.06	0.57	6.88	1.7	8.28	0.62	6.37	1.2	4.94	0.72	3.5	0.54	
95.086	Terpenes fragments	$C_7H_{11}^+$	1.42	0.98	22.01	4.07	118.99	51.96	37.93	3.09	17.63	2.88	16.58	1.41	
97.029	2-Furfural	$C_5H_5O^+$	0.83	0.51	4.34	2.33	6.36	0.81	5.65	2.19	3.29	0.79	2.85	0.1	§
97.101	Fragments	$C_7H_{13}^+$	0	0	3.67	0.74	5.11	1.24	3.19	0.71	1.11	0.17	1.67	1.16	
99.080	cis-3-Hexenal/2-Hexenal	$C_6H_{11}O^+$	0	0	3.61	1.25	5.35	2.9	6.2	0.43	2.36	0.66	4.22	0.45	§
101.023	n.i.	$C_4H_5O_3^+$	7.89	1.18	7	1.27	14.65	1.88	30.71	7.9	7.45	2.3	11.46	0.9	
101.059	n.i.	$C_5H_9O_2^+$	3.28	1.28	3.35	0.63	4.92	0.54	7.11	2.65	3.03	1.21	3.99	0.92	
101.096	Hexanal	$C_6H_{13}O^+$	3.44	0.58	3.98	0.72	6.05	0.59	17.81	3.43	4.1	0.58	5.66	0.94	&
103.101	Ethyl propionate	$C_5H_{11}O_2^+$	15.37	3.28	24.18	4.43	51.08	6.36	101.95	15.83	24.65	3.3	32.66	1.59	
105.035	n.i.	$C_4H_9OS^+$	4.75	2.67	6.72	2.97	7.58	2.16	10.06	4.54	6.68	2.76	4.83	1.72	
105.069	2-Phenylethyl acetate fragment	$C_8H_9^+$	67.53	5.11	99.58	22.94	198.86	23.44	200.15	25.74	97.31	7.88	127.96	7.64	^
107.089	Benzaldehyde	$C_7H_7O^+$	1.5	0.37	1.39	0.2	3.45	0.9	3.32	1.01	1.25	0.29	1.75	0.54	&
109.085	Terpenes fragments	$C_8H_{11}^+$	0	0	2.28	0.62	10.86	2.04	5.79	0.8	2.16	0.13	2.5	0.07	
111.08	5-Methylfurfural/1-(2-Furyl)ethanone	$C_6H_7O^+$	0	0	1.12	0.41	1.79	0.81	1.88	0.63	1.65	0.26	1.4	0.24	&
113.065	(E)-2-heptenal	$C_7H_{13}O^+$	0	0	2.33	1.15	0.83	0.23	1.46	0.31	0	0	0	0	§
115.067	Heptanal/2-Heptanone	$C_7H_{15}O^+$	0	0	2.64	1.11	2.19	0.63	2.13	0.46	0.82	0.16	1.25	0.57	&
117.095	Ethyl 2 - methylpropanoate/ Isobutyl acetate	$C_6H_{13}O_2^+$	63.85	19.89	82.31	24.8	114.41	29.24	109.96	25.05	67.67	23.29	84.92	13.29	#
119.088	Terpenes fragments	$C_9H_{11}^+$	0	0	1.27	0.46	1.6	0.37	1.74	0.55	1.52	0.37	1.54	0.32	
121.101	2-Phenylacetaldehyde	$C_8H_9O^+$	1.48	0.34	1.36	0.36	2.8	0.33	3.23	0.52	1.1	0.33	1.86	0.19	X
123.116	2-Phenyl ethanol/ terpenes fragments	$C_8H_{11}O^+$	0	0	1.23	0.46	3.62	0.68	1.57	0.14	1.28	0.37	1.13	0.17	-
125.101	n.i.	$C_8H_{13}O^+$	0	0	1.51	1.03	1.81	0.36	1.28	0.15	0.84	0.12	1.59	0.4	
127.112	(E)-2-octenal	$C_8H_{15}O^+$	0	0	2.21	1.23	4.77	1.55	3.31	0.7	1.8	0.35	2.19	0.14	§
129.092	1-Octanal	$C_8H_{17}O_2^+$	0	0	1.35	0.58	1.38	0.63	1.56	0.51	1.61	0.13	1.54	0.3	&
131.108	Isoamyl acetate/Ethyl 3 - methylbutanoate	$C_7H_{15}O_2^+$	0.94	0.39	3.34	1.17	4.45	0.54	6.23	0.89	3.21	0.71	3.17	0.43	*
133.107	p-cymenene [20*]	$C_{10}H_{13}^+$	0	0	2.31	0.49	1.31	0.32	1.66	0.21	0.98	0.11	0	0	
135.117	α-Cymene	$C_{10}H_{15}O^+$	0	0	1.29	0.5	1.52	0.21	1.03	0.07	1.02	0.33	1.03	0.28	
137.132	Monoterpenes (i.e. Myrcene, limonene)	$C_{10}H_{17}O^+$	0	0	3.36	1.27	11.7	2.75	2.72	0.23	1.34	0.53	1.87	0.36	#
141.116	Terpenes fragments	$C_{10}H_{21}^+$	0	0	0	0	1.74	0.72	0	0	0	0	0	0	
143.122	2 - Nonanone/ Nonanal	$C_9H1_9O^+$	1.41	0.26	2.30	1.20	3.64	1.95	4.35	1.89	4.52	1.43	3.29	1.42	#
145.127	Ethyl caproate/ isobutyl isobutyrate	$C_8H_{17}O_2^+$	14.3	5.04	82.37	22.14	85.56	15.77	84.38	7.4	38.18	5.88	71.25	11.15	*

147.080	n.i.	$C_{11}H_{15}^+$	0	0	26.5	13.9	34.66	17.22	36.86	31.39	19.21	6.28	4.69	1.78	
151.122	Myrtenal/Perillene/3-Nopinenone	$C_{10}H_{15}O^+$	0	0	1.46	0.32	1.1	0.44	1.22	0.49	0	0	0	0	
153.132	(E,E)-2,4-decadienal/ Oxigenated terpenes	$C_{10}H_{17}O^+$	0	0	1.19	0.49	1.48	0.27	1.48	0.75	1.07	0.45	1.01	0.21	§
157.155	Decanal/Citronellol	$C_{10}H_{21}O_2^+$	0	0	0	0	1.03	0.33	0	0	0	0	1.1	0.21	&
159.146	C9 ester (i.e. Heptyl acetate)	$C_9H_{19}O_2^+$	0	0	1.38	0.58	1.41	0.46	2.14	1.08	1.33	0.56	1.09	0.27	&
173.159	Ethyl caprylate/Ethyl octanoate	$C_{10}H_{21}O_2^+$	0	0	2.07	0.5	3.09	0.66	3.03	0.62	2.71	0.37	3.63	0.36	*
	Total VOCs (ppbv)		19814.4	4401.9	20731.4	2319.2	31535.5	4134.7	35874.8	4567.53	20442.6	2311.9	26575.9	3031.4	
	Total number of compounds		50		70		73		71		67		67		

In general, the hops beer compared to the "reference beer" showed a greater presence of compounds such as: (1) terpenes compounds and their fragments (i.e. m/z 95.86,97.101, 137.132); (2) C6 compounds (i.e. m/z 81.069, 99.080, 111.080), and (3) Ester (i.e. m/z 89.059, 145.122, 159.146) as reported in Table 3. These results are in agreement with previously studies [31, 32] that some terpene and several ester compounds in beer derived by hops are main contributors on hop varietal aroma.

On the contrary, the amount of sulphur compounds (detected at m/z 49.011, 63.027, 77.038 and 91.024) appear to be unaffected by the addition of hops during the dry-hopping phase. These, compounds which when present at too high levels, negatively affect the beer flavor; if they are present in the right doses contribute to the typical character of lagers (bottom-fermented beers) [25]. For example, dimethysulphide (DMS) (m/z 63.027) is an important compound for the aroma and flavor of beer. As reported by Landaud et al. [26], a good odor threshold level of DMS is between 30 and 100 ppb, while when present at a concentration higher than 100 ppb, DMS may impart a usually undesirable flavor described as "cooked sweet corn" [27].

Principal components analysis (PCA) was performed to analyse the differences in volatile compounds according to each beer type (Figure 2.). Loadings of the PCA are reported in Figure 3. The three main components accounted for 70.9 % of the variability of the original data. By the PCA, the beers obtained with Cascade (a commercial hop; positive side of the PC1) are separated from the ones obtained with wild hops, which are located on the negative side of the first component. The second axis allow the distinction of the CASC_T and CASC_S beers (positive side of PC2) from CASC_C and RIF (negative side of PC2). On the first axis, VOCs are mainly positioned on the positive side; for this reason, beers positioned on the left side of Figure 2. showed generally reported higher values of volatile compounds.

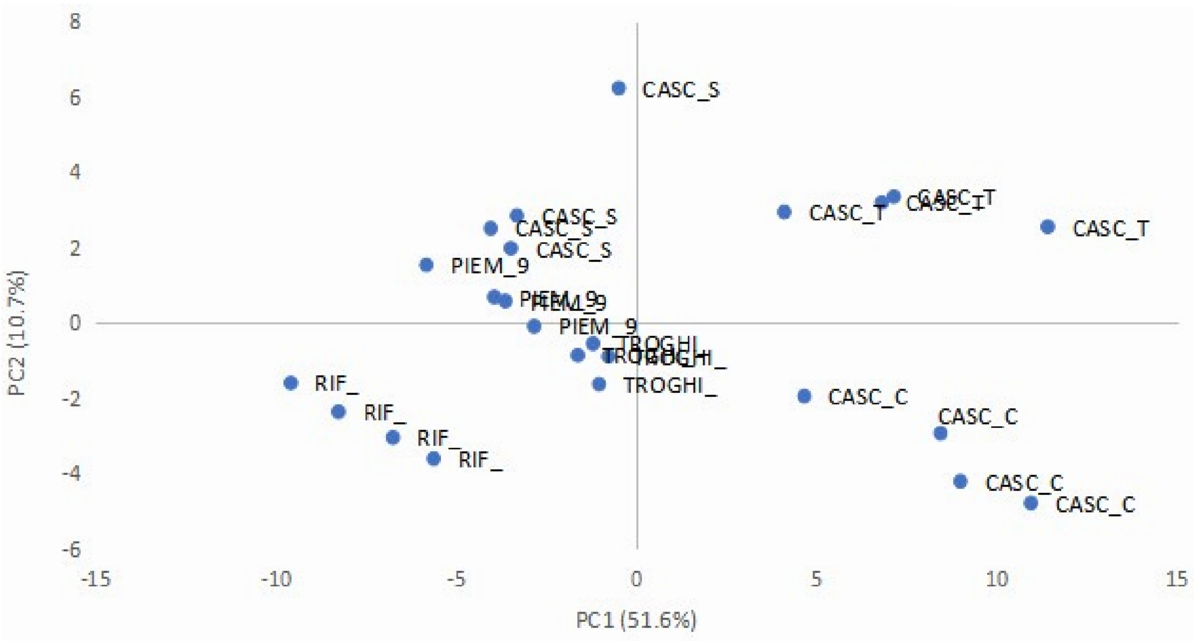

Figure 2. Plot of the first two axes of the PCA conducted on the VOCs of the different beers

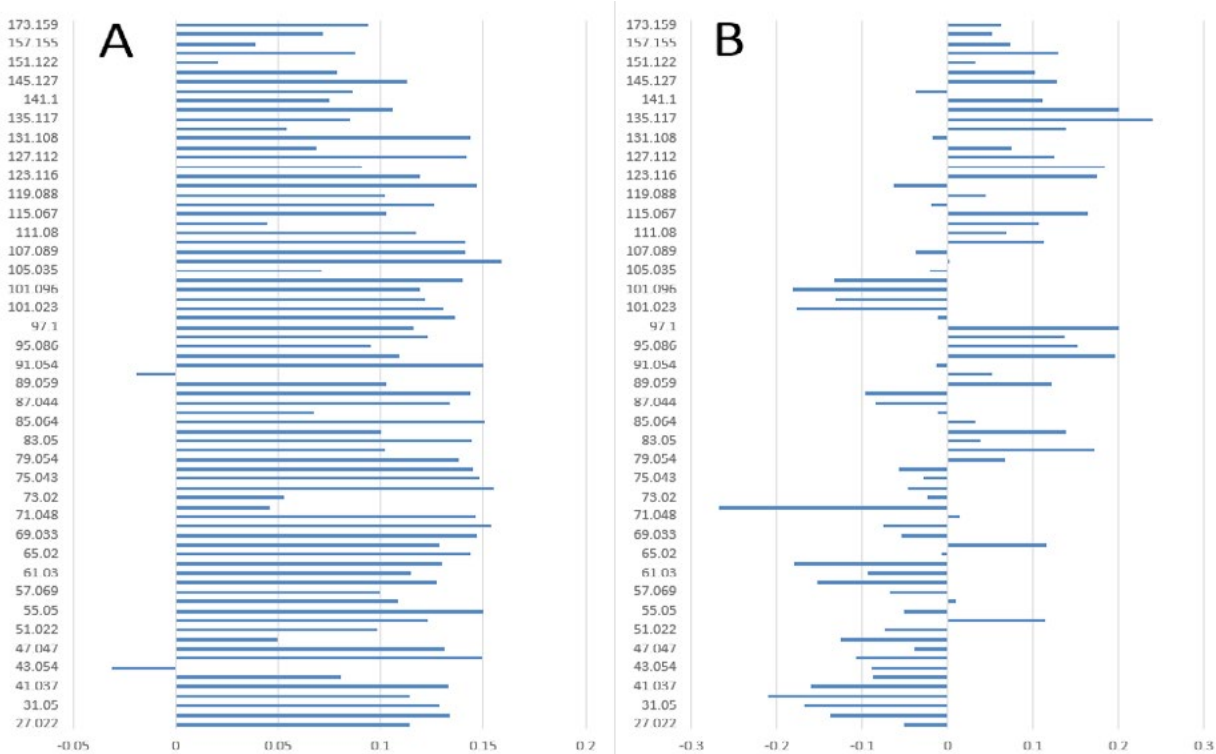

Figure 3. Loadings of of the first two axes of the PCA conducted on the VOCs of the different beers. A = PC1; B= PC2

3.2 Sensory evaluation

The sensory evaluation has been structured into two section regarding respectively the beers aromatic profiles and their taste.

3.2.1 Panel test

The results of the "beer sommelier" after tasting the five craft beers were presented in Table 1. The spider-plot diagram showed the judgment of eleven attributes taken into consideration (Figure 4). As expected, reference beer (not hopped) stand out with a clear pick toward cereal and minimun/no scores with regards to all the other attributes. The wild hops collected ("Piemonte 9" and "Troghi") show a similar restrained aroma with a low to moderate intensity and low sweet, resinous and herbal character. The "Cascade commercial" seems to impart the strongest aroma with the highest citrusy and spicy attributes. "Cascade from Travalle", even if with a lower intensity, produced an its own aroma profile showing the strongest floral and tropical notes.

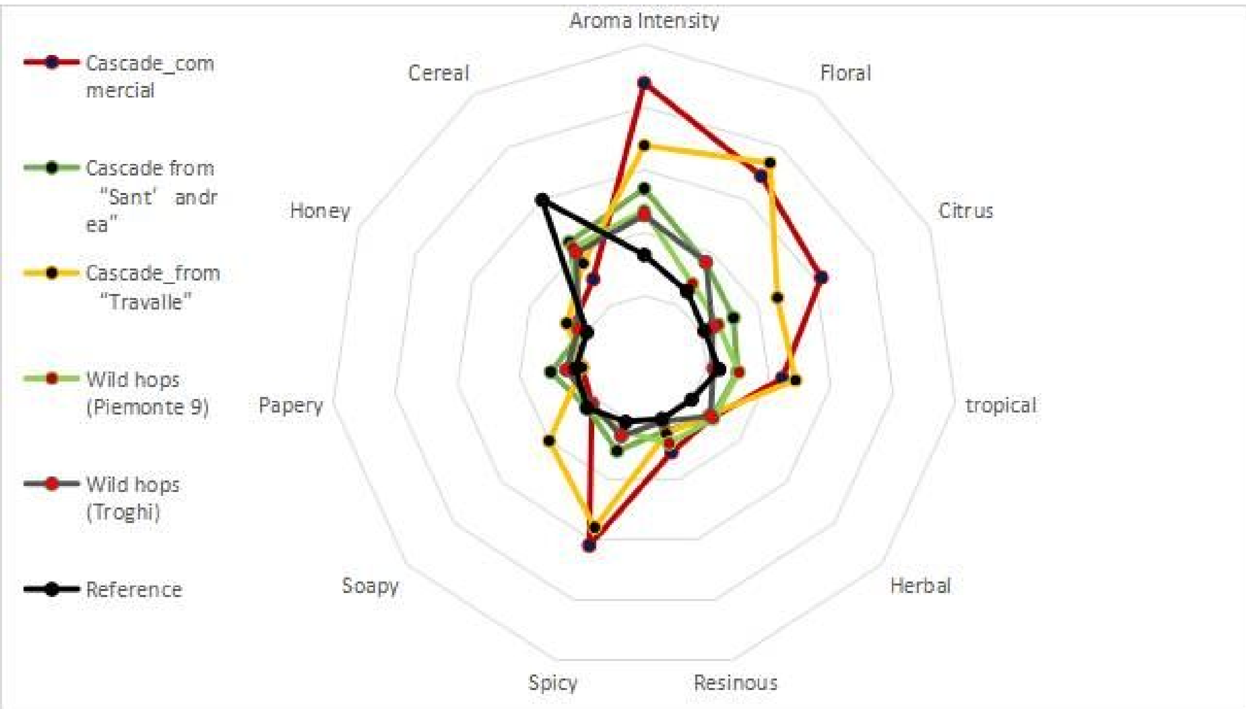

Figure 4. Aroma evaluation: Schematic representation of the average values (five-point intensity scale) of the scores of the 11 attributes selected and obtained by the six beers compared

The results obtained from the taste evaluation do confirm the aroma analysis. Indeed, the "Cascade commercial" does score the highest taste intensity (3.5) tailed by the "Cascade from Travalle". The others do follow far behind. Concerning bitterness, the same trend is only true for the the attribute itself but not for its persistence where "Cascade_from Travalle" resulted to be milder (2.52 vs. 3.20). The same last beers appear to impart a tiny bit of sourness. Instead, it is interesting to notice how the "Cascade_from Travalle" produced a bit of astringency more than its commercial rival.

3.2.2 Consumers' acceptability

The world market of beers and craft beers is characterized by similar quality requirements, even if different parameters can drive the customer preferences. It was observed that craft beer is chosen according to different flavour preferences compared to commercial beer, it is mainly drunk by frequent beer drinkers in pubs and with family members and it is perceived to be of higher quality than commercial beer due to the raw materials used for brewing and its overall quality [28]. Aquilani and co-author [28] reported many commercial beer attributes (i.e. aroma, color, carbonation, etc.) that can be used as potential drivers towards the consumption of craft beer, among these aromas and flavor which play an important role. The consumer test is a method of investigation used in sensory sciences. It is normally carried out through an analysis of the consumers 'perception, following their acceptance in the assessment of a product (beer). Regarding the consumer test, significant differences ($p = 0.01$) were registered (Table 2.). The consumer acceptance was calculated as the percentage of respondents who liked the sample, with scores >5 and it has been observed that, the 91% of consumers expressed a positive opinion for the beers namely "Cascade commercial", followed by "Cascade from Travalle" 85% and "Cascade from Sant'Andrea" 62% (Table 2.). Significant difference (ANOVA test) was observed between Commercial Cascade, "Cascade from Travalle" versus wild hops and reference beer (Table 2.). On the contrary, the beers produced without hops or using wild hops, were not accepted. Therefore, the beers preferred by the consumers were those produced using selected hops, both, commercial and grown in Italy.

3.3 VOCs and sensory interaction

Figure 5. shows the CANOCO output for the analysis of correspondence of VOCs and the consumer test flavour variables. As it can be observed, on the positive side of the first axis the CASC_C beer is positioned in contraposition to the other beers. The main contribution on the positive side of the PC1 (flavour loadings) is due to resinous, citrus and tropical and a negative high contribute to papery flavour. On the positive side of the second axis, in contraposition to all the other beers, CASC_T beer is positioned. The main positive contribution to PC2 is given by herbal, spicy and floral. On the first quadrant, it could be observed a high contribution of persistence and intensity. Flavour loadings more appreciated

by consumers (i.e., the ones positioned on the first quadrant) are in contraposition with cereal and papery flavours. These last are highly present in PIEM_9, TROGHI_ and CASC_S, positioned on the negative side of both axes together with the reference beer (RIF_) which reported low levels of the other flavours' loadings.

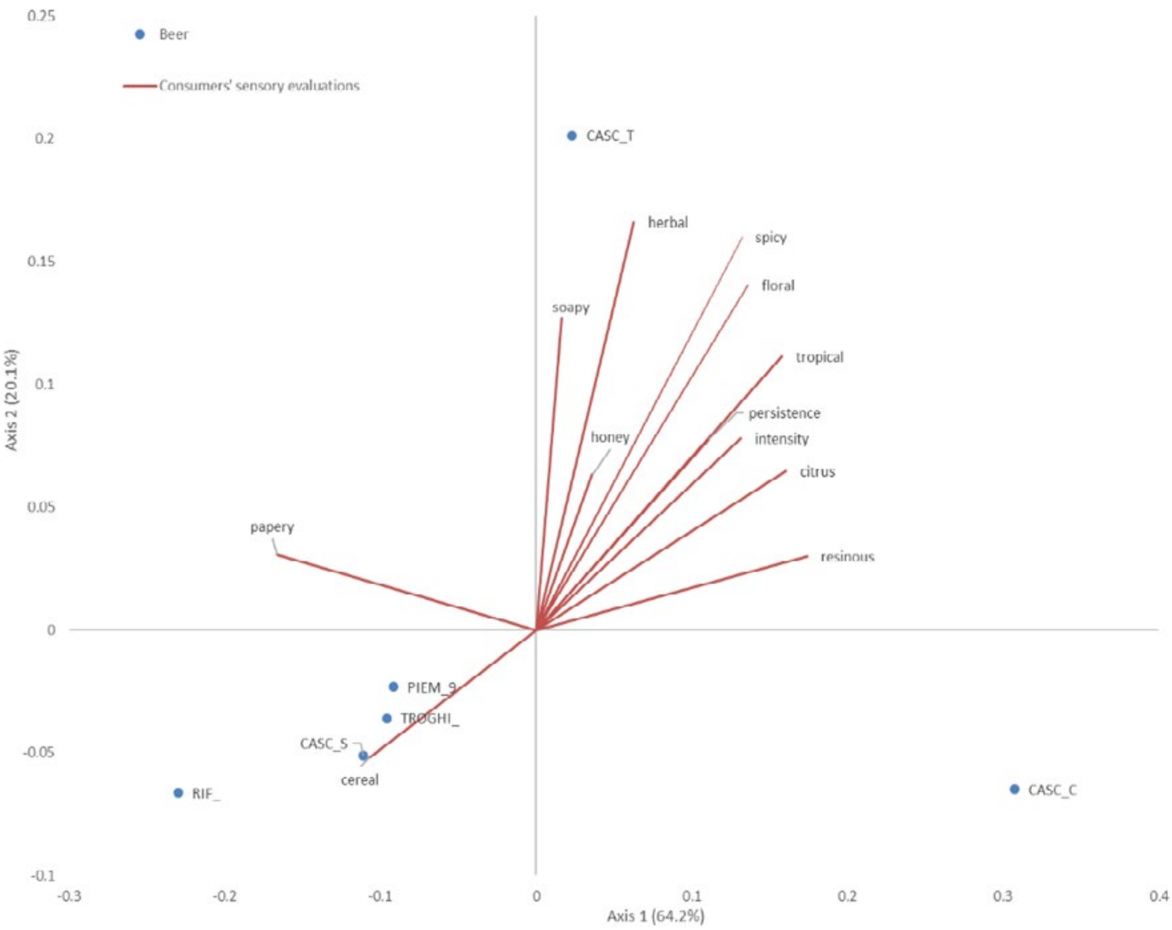

Figure 5. CANOCO output for the analysis of correspondence of VOCs and the consumers' sensory evaluations variables. Beers and consumers' sensory evaluations variables loadings (expressed as vectors) plot

Figure 6. shows the CANOCO output for the analysis of correspondence of VOCs and the consumers' sensory evaluation variables. The results represent the VOCs, beers and consumers' sensory evaluations variables loadings which are expressed as vectors. The first axis appears to be characterized mainly by the aromas spicy, floral, tropical, resinous, citrus. Such aromas are paired with the following VOCs: 31.02, 59.049, 61.03, 101.023, 101.096, 103.1, 151.122 and 159.146. For detailed information please consult Table 3. reporting the complete VOCs scores.

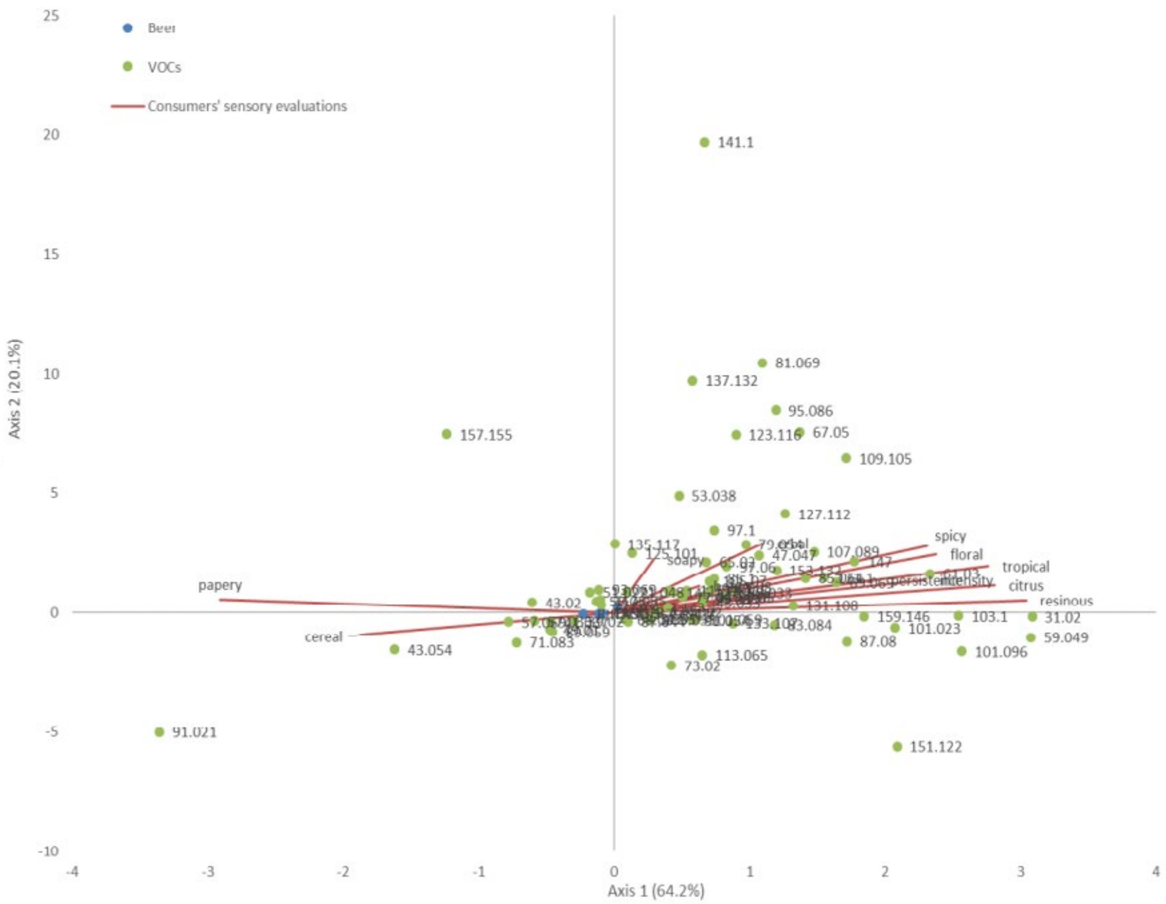

Figure 6. CANOCO output for the analysis of correspondence of VOCs and the consumers' sensory evaluations variables. VOCs, beers and consumers' sensory evaluations variables loadings (expressed as vectors) – triplot

4. Conclusion

The work investigated the relationship among VOCs and sensory properties in craft beer produced using two selected wild hop varieties against a commercial hop grown in the same sites in comparison to standard trying to individuate new interesting aroma varieties. Despite the preselection of the wild hop genotypes, the results produced by both, consumer and panel tests, show low interest for the aromas the hops imparted to the beers produced in relationships to the commercial variety grown and bought. This points out some limitations to this kind of approach like the need to embrace several more varieties, to plan a longer screening campaign and the budget needed to set up and carry out a more comprehensive and exhaustive study.

The grown commercial cascade resulted to be interesting, producing a modified aroma profile when compared to its commercial counterpart. This represent a potential for the production of hops with characterized aroma grown in different region. The headspace analysis by PTR-ToF-MS was helpful in differentiating and quantify compounds that play an important role in characterize the flavor profile of each beers studied.

However, the history teaches us as even with much less efforts it is possible to determine great practical implications, this is the story of Amarillo Hops, a citrusy wild variety discovered by accident in 1990 near Washington.

Future studies will point to screen other wild genotypes to identify new hops for direct use or breeding when interesting e.g. for just one compound or other characteristics. One interesting path might be to evaluate the cultivars with interesting (even if not enough) quality and to program a wild strain research on the base of the habitat and average soil properties.

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Conflict of interest

The authors declare no competing financial interest.

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