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The Comparison and Brewing Value of Saaz Hop Pedigree

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Abstract: The well-known hop variety Saaz, which gives the Pilsner lager beer characteristic hop aroma may be threatened by climate change in the future. Therefore, new Saaz-related hop varieties, Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz Shine were bred recently. To evaluate whether these varieties are acceptable for traditional lagers, their comparison was carried out. For this purpose, sensorial and chemical analyses of hops and related beer including namely analysis of hop resins and hop oils were performed. Sensory profiles of Saaz varieties are very similar (fine hoppy aroma, floral, herbal) except for Saaz Comfort which has a little higher aroma intensity, and Saaz Shine which has the most noticeable fruity scent with traces of citrusy of all. Also, the chemical profiles are very close, α-humulene, β-pinene, (E)-β-farnesene, β-caryophyllene, and myrcene are the most abundant. As a result of decoction mashing and kettle-hopping technology with bottom fermentation, are very similar lager beers with hoppy, floral, herbal, fruity, and spicy aromas. Typical hop oils are farnesol, linalool, methyl geranate, β-pinene, and limonene. The high concentration of farnesol in beer correlates with the concentration of (E)-β-farnesene and farnesol in hops. New Saaz varieties are well used for the production of the Pilsner type of lager without affecting the traditional sensory aroma of this widespread style. Varieties have a higher yield and concentration of bitter acids and hop oils. Moreover, Saaz Shine and Saaz Comfort have very good resistance to drought, which is an important property from a climate change perspective.

Keywords: Hops; Saaz; lager; hop oils; projective mapping

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1. Introduction

Hops are one of the most important raw materials of beer because of their bitter and aromatic properties. Hop breeding in the Czech Republic, mostly focuses on fine-aroma hops. The Saaz fine aroma variety bred by Oswald is the most well-known one in the world [1].

In 2011, the fine aroma Saaz Late variety, relating to the original Saaz, was registered [2]. Subsequently, in 2019, three new varieties Saaz Brilliant, Saaz Comfort, and Saaz Shine were registered [3]. These varieties show several identical features with Saaz, therefore, a complex and long-term study describing their pedigree and breeding methods, and comparing hop resins content, hop oils profile, and aroma yield was carried out [4].

Few articles have dealt with the Saaz variety up to now. Four hop varieties were compared, namely, Target, Saaz, Hallertauer Hersbrucker, and Cascade. Odor-active compounds were identified using GC-MS (Gass Chromatography with Mass Spectrometry). The authors focused mainly on substances causing a "noble" aroma, often described as herbal and spicy [5].

The terpenoid metabolomic pattern of hop-essential oil that derived from the Saaz variety was performed using the headspace solid-phase microextraction combined with GC-MS. The authors identified 27 terpenoid metabolites with dominating monoterpenes and sesquiterpenes followed by

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oxygenated monoterpenes and hemiterpenes. Especially, myrcene, α-humulene, and β-caryophyllene, which together represent about 80% of the total volatile fraction from the hop-essential oil were the main compounds. The hop aroma of the Saaz variety was described as "very mild with pleasant hoppy notes" [6].

Another study mentioning odor-active compounds in Saaz compared strongly hopped beers with Saaz, Hersbrucker, and Cascade varieties with unhopped beer using GC-O (GC with Olfactometry) and sensory evaluation. According to this study, citrus and floral notes characterized the hop aroma of Saazer beer. Linalool, geraniol, and β-ionone were shown to contribute to the floral note based on the Charm values and the aroma values [7].

Saaz and Challenger hop pellets were used for analysis of beer aromas that derived from hops using sensorial AEDA (Aroma Extract Dilution Analysis). The identification of hop aromatic compounds in beer was performed by GC-O. Beer brewed with a late kettle addition of Saaz hops was described as "fruity (citrus), flowery, spicy, fresh, beer-like" and more pleasant in comparison with Challenger. One spicy/hoppy compound, unmodified from hop to beer, proved responsible for the most intense odor in both hopped beer extracts. However, the chemical structure of this compound has not been revealed [8].

The impact of seven Slovenian, one American, and four Czech hop varieties including Saaz in single-hopped beers (both kettle-hopped and dry-hopped) was evaluated using GC-MS and sensorial analysis. Dominant compounds in Saaz hop were myrcene, (E)-β-farnesene, and α-humulene, whereas limonene, linalool, farnesol, myrcene, (E)-β-farnesene, and α-terpinol were major hop oils in kettle-hopped beer [9].

The recent study focusing on pilot brewing tests of the three Saaz varieties (S. Brilliant, S. Shine, and S. Comfort) monitored sensory profiles of kettle and kettle+dry single-hopped pale lager beers. While kettle+dry beers hopped by new Saaz varieties were distinguished from beer hopped with original Saaz in the triangle test, kettle-hopped beers were very similar except for S. Comfort [10]. Similarly, in a previous study, S. Late did not differ from Saaz when kettle-hopped beer was prepared [2].

The study aimed to reveal and compare the impact of five Saaz-related varieties in kettle-hopped beer which was prepared by traditional technology of Pilsner lager beer, also by double decoction mashing, kettle-hopping, bottom fermentation, and cool and long lagering. The basic sensorial and chemical analyses of hops and beer were performed according to well-known methods. Changes in a hop oil profile during wort boiling and fermentation were discussed based on a previously described chemical transformation.

2. Materials and Methods

2.1. Chemicals

Standards of α-pinene (99.0%), β-pinene (99.0%), isobutyl isobutyrate (98.0%), isoamyl isobutyrate (98.0%), myrcene (75.0%), limonene (97.0%), ocimene (90.0%), linalool (97.0%), methyl hexanoate (98.0%), methyl heptanoate (99.8%), methyl octanoate (99.8%), methyl nonanoate (99.8%), methyl decanoate (99.0%), 2-nonanone (99.0%), 2-decanone (98.5%), 2-undecanone (99.0%), 2-dodecanone (98.5%), 2-tridecanone (97.0%), (E)-β-farnesene (90.0%), α-humulene (90.0%), β-caryophyllene (98.0%), methyl geranate (AldrichCPR), α-terpineol (97.0%), terpinen-4-ol (95.0%), geranyl acetate (99.0%), (Z)-geraniol (nerol) (90.0%), α-ionone (90.0%), β-ionone (95.0%), α-irone (90.0%), β-caryophyllene oxide (98.0%), farnesol (90.0%), and 1-hepten-3-ol were purchased from Sigma-Aldrich (Germany). Dichlormethane, n-hexane, and acetonitrile of reagent grade were obtained from Honeywell (USA). Deionized water was prepared by Milli-Q system (Millipore, USA).

2.2. Hop sample analysis

Hops were homogenized during post-harvest processing and six independent samples of each variety were sampled and finely grounded. Hop oils were extracted from hop samples by solid-liquid extraction. A portion of 50 mg of a grounded sample with 5 μl of internal standard 1-hepten-3-ol (7.8

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g/L) was mixed with 400 µl of dichloromethane:acetonitrile (2:1, v/v) solution. The mixture was heated at 50 °C for 60 min, then cooled down and finally transferred into a vial containing 400 µL of water to wash the extract from highly polar interferents by liquid-liquid extraction for 1 min (three times). The phases of water and organic extract were separated by centrifugation (3 min, 1200 rpm).

The organic extract was analyzed by heartcut GCxGC method using a gas chromatograph Agilent 7890 B equipped with LTM column modules, the Deans-switch system, FID, and QQQ MS 7000D (Agilent Technologies. Santa Clara. USA). Separations were primarily carried out on DB-5MS UI (15 m x 0.25 mm x 0.25 µm. 5%-phenyl-dimethylpolysiloxane) connected to MS detection. Heart-cut aliquots performed by the dean-switch system were sent to the second column HP-INNOWAX (30 m x 0.25 mm x 0.25 µm, polyethyleneglycol) connected to FID. The sample injection of 1µl volume was performed in a split mode (10:1) at 250 °C. Separation on the first column was performed with a ramped flow: 4 mL/min (1 min) – 100 mL/min2 – 2 mL/min (0.6 min) – 100 mL/min2 – 1 mL/min (14.5 min) – 100 mL/min2 – 4 mL/min (1.5 min) of helium (Air Products, the Czech Republic), and gradient temperature program was 60 °C (1 min) – 200 °C/min – 80 °C (0.5 min) – 50 °C/min – 110 °C (1 min) – 25 °C/min – 140 °C (1 min) – 25 °C/min – 160 °C (3 min) – 25 °C/min – 180 °C (1 min) – 25 °C/min – 260 °C (0 min) – 50 °C/min – 300 °C (2 min). Separation on the second column was maintained at the constant pressure of 42 psi and the temperature program: 50 °C (8 min) – 20 °C/min – 100 °C (1 min) – 30 °C/min – 255 °C (1 min). MS detection was performed in the dMRM mode (see Supporting Information S1) at standard ion source conditions (70 eV, 230 °C). The flame-ionization detection was maintained at 270 °C, flow rate of air, hydrogen, and nitrogen were 400, 30, and 25 mL/min, respectively.

2.3. Brewing test

The beer samples were prepared in 250 L the research brewhouse of Kaspar-Schulz (Germany). The grist composition for each brew was 33 kg of Pilsner malt (Benešov, the Czech Republic) with extract-dry basis 81.5% and color 4.2 units EBC. The double decoction mashing regime was used with a mash-in temperature and the mash-out temperature of 46 and 75 °C, respectively. The maximum turbidity of sweet wort was set to 20 EBC and the last running to 50 EBC. The volume of sweet wort before boiling was 210 L. Single kettle hopping with 100% of the tested hop variety was carried out in three doses: 30% (at the beginning), 40% in the 20th minute, and 30% 15 minutes before the end of the 75 min wort boiling.

Beer samples were fermented identically at 12 °C with yeast W34/70 from Fermentis in cylindroconical tanks for seven days. The maturation took place at 2 ± 0.5 °C for 21 days. Finally, the samples were filtered on a plate filter with S10N filter plates (Hobra Školník, Broumov, the Czech Republic), and bottled without access to oxygen.

The experiment was performed twice and the results were processed together.

2.4. Beer sample analysis

Hop oils were extracted from beer by steam distillation (Büchi, Distillation Unit K-350. Switzerland): 2 x 50 mL of beer samples containing 250 µL of internal standard 1-hepten-3-ol (100 mg/L) were distilled for 4 min. Subsequently, the obtained distillate was shaken with 10 ml of a mixture of dichloromethane:hexane (1:1, v/v) at 250 rpm for 1 h. The mixture was cooled down to 4–6 °C to allow full separation of water and organic extract which was analyzed by the previously described GC-MS method [11].

The determination of the original extract, alcohol, bitterness, and color and α- and β-acids in hops was performed according to the EBC methods [12–16].

2.5. Sensory analysis of beer

Sensory analyses were carried out by a professional 12-member sensory panel. The assessors were selected and trained according to ISO 8586:2015 and ISO 11132:2012. The sensory evaluations were conducted in a sensory laboratory equipped according to ISO 8589:2008. The assessors were

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acquainted and trained using certified beer flavor standards (FlavorActive™, Great Britain). The beer samples were served in glass cups in volumes of about 100 mL at 10 ± 2 °C.

The sensory profile of beer was evaluated using basic parameters of beer such as fullness, bitterness, astringency, sourness, sweetness, hoppy, estery, and yeasty on a six-point scale (intensity: 0 – no, 1 – very low, 5 – very high). Assessors had to specify in detail the intensity of particular hop aromas such as hoppy, fruity, citrusy, floral, spicy, herbal, and woody.

The method of projective mapping was performed in two sessions (each repetition of the brewing test in one session). The assessors obtained six samples (five tested and one control, i.e. one randomly selected sample) with randomly assigned codes and were asked to arrange the samples on white paper based on their mutual sensory similarity/dissimilarity – similar samples were placed closer to each other than dissimilar samples. The assessors also characterized each sample by a short description. The final arrangement of samples in the plane was transformed to x and y coordinates (the lower left corner of the paper was set as the origin). Such obtained data were processed by Generalized Procrustes Analysis (GPA) in XLSTAT software (version 2021.4.1. Addinsoft).

Concentrations of hop oils in hop and beer samples were evaluated by principal component analysis (PCA) to visualize samples in multivariate space (Rstudio, version 1.1.456. with R v 4.0.1) by FactoMineR (version 2.3), factoextra (version 1.0.7).

3. Results and Discussion

The basic parameters of the GC-MS method for analyzing hop oils in hops are given in Table 1.

Table 1. Validation parameters of heart-cutting GC method for determination of hop oils in hop and beer.

Essential oil	Absolute Recovery	Correction Factor	LOD	LOQ	Relative Uncertainty
	(%)		(mg/kg)	(mg/kg)	(%)
Isobutyl isobutyrate	87.3	-	0.98	3.25	20.0
Methyl hexanoate	87.7	-	0.16	0.54	25.0
α-Pinene	78.9	-	1.42	4.75	25.0
Isoamyl isobutyrate	98.6	-	0.30	1.01	25.0
Methyl heptanoate	96.0	-	0.60	2.00	25.0
2-Nonanone	157.3	0.64	0.70	2.34	25.0
Linalool	166.8	0.60	0.94	3.13	15.0
Methyl octanoate	93.9	-	0.79	2.64	25.0
Methyl nonanoate	88.9	-	1.02	3.41	25.0
(Z)-Geraniol (Nerol)	101.7	-	1.48	4.92	25.0
(Z)-Methyl geranate	96.5	-	0.88	2.94	10.0
2-Undecanone	94.0	-	2.29	7.64	10.0
Geranyl acetate	96.7	-	1.92	6.39	25.0
2-Dodecanone	105.8	-	0.96	3.20	15.0
α-Ionone	107.7	-	2.23	7.42	25.0
β-Caryophyllene	70.0	1.43	1.73	5.76	10.0
(E)-β-Farnesene	82.0	-	1.76	5.86	10.0
α-Irone	116.0	-	0.53	1.77	25.0
β-Caryophyllene oxide	96.8	-	1.78	5.92	25.0
Farnesole	91.4	-	0.55	1.83	15.0
β-Pinene	51.4	1.94	1.38	4.59	20.0
Myrcene	73.7	1.36	0.71	2.38	25.0
Limonene	80.5	-	0.52	1.74	25.0

(E)-β-Ocimene	81.1	-	0.30	1.00	25.0
2-Decanone	119.5	-	2.53	8.45	10.0
Terpinen-4-ol	100.4	-	1.29	4.30	25.0
α-Terpineol	96.1	-	1.61	5.35	25.0
Methyl decanoate	98.4	-	0.62	2.07	10.0
(E)-Methyl geranate	98.6	-	0.67	2.22	15.0
α-Humulene	86.5	-	0.52	1.74	20.0
2-Tridecanone	101.6	-	1.51	5.03	25.0
β-Ionone	102.3	-	0.85	2.85	25.0

The used heartcut GCxGC enables a wider linear operating range (up to 107) [17]. The concentrations of hop resins are given in Table 2.

Table 2. Hop resin content in studied beer samples.

Variety	Cohumulone (% rel.)	Colupulone (% rel.)	α-acids (wt%)	β-acids (wt%)	α/β	X (wt%)	DMX (wt%)
Saaz	20.59	40.66	3.84	3.94	0.97	0.34	0.06
S. Late	22.93	40.18	2.64	4.22	0.63	0.30	0.03
S. Brilliant	21.01	42.62	3.42	2.30	1.49	0.09	0.02
S. Comfort	18.94	42.91	3.95	3.49	1.13	0.35	0.08
S. Shine	20.92	46.79	3.42	2.73	1.26	0.33	0.02

The highest concentration of α-acids has S. Comfort 3.95 wt%, however, it has the lowest concentration of cohumulone (18.94% rel.). It is reflected in the lowest final bitterness of tested beer hopped by S. Comfort (see Table 3).

Table 3. Basic parameters of single-hopped beers.

	Saaz	S. Late	S. Brilliant	S. Comfort	S. Shine	Saaz
Original extract (% w/w)	12.6 ± 0.2	12.4 ± 0.1	12.5 ± 0.0	12.3 ± 0.1	12.6 ± 0.1	12.6 ± 0.2
Alcohol (% v/w)	5.0 ± 0.2	5.0 ± 0.2	5.1 ± 0.0	5.0 ± 0.1	5.1 ± 0.2	5.0 ± 0.2
Alcohol (% w/w)	3.9 ±0.2	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.2	3.9 ±0.2
Apparent Extract (% w/w)	3.2 ± 0.2	3.0 ± 0.2	2.9 ± 0.0	3.0 ± 0.1	3.2 ± 0.4	3.2 ± 0.2
Real Extract (% w/w)	5.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.0	4.8 ± 0.1	5.0 ± 0.3	5.0 ± 0.1
Apparent Attenuation (%)	74.4 ± 2.0	75.5 ± 1.7	76.6 ± 0.1	75.8 ± 0.1	74.7 ± 3.1	74.4 ± 2.0
Real Attenuation (%)	60.1 ± 1.6	61.0 ± 1.4	62.0 ± 0.0	61.0 ± 0.5	60.0 ± 2.5	60.1 ± 1.6
Bitterness (BU)	39 ± 1	41 ± 1	37 ± 1	36 ± 1	37 ± 2	39 ± 1
Color (u. EBC)	8.9 ± 0.4	9.5 ± 0.1	9.2 ± 0,0	8.5 ± 0.1	9.4 ± 0.3	8.9 ± 0.4

mean value of six measurements ± standard deviation.

This phenomenon was described in several previous studies. In 1972, Rigby studied the utilization of individual homologs of α-acids [18]. According to this study, cohumulone is utilized more efficiently than humulone. Further, Ono et al demonstrated that during wort boiling the relative amount of formed isocohumulone was significantly higher in comparison with isohumulone and isoadhumulone [19]. Moreover, the relative amount of isocohumulone lost during fermentation is lower than that of isohumulone and isoadhumulone. Irwin et al and Jacobsen et al concluded likewise [20,21]. Irwin et al. published that cohumulone is better utilized than humulone or adhumulone, probably due to higher losses of humulone and adhumulone in the kettle and of isohumulone and

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isoadhumulone in the fermenter [20]. More recently, Jaskula et al and Protsenko et al. determined this phenomenon using a detailed kinetic study [22,23].

The next significant extreme among studied Saaz varieties is a high concentration of β-acids in S. Late (4.22 wt%) reflecting a very low ratio of α/β (0.63). The highest ratio α/β has S. Brilliant (1.49), which is more than doubled. The main trends are in good correlation with long-term data described previously [4].

Concentrations of particular hop oils are given in Table 4.

Table 4. Hop oils profile in studied samples of Saaz varieties.

	Saaz	**S. Late**	**S. Brilliant**	**S. Comfort**	**S. Shine**
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Isobutyl isobutyrate	2.04 ± 0.18	3.26 ± 0.11	3.27 ± 0.15	3.16 ± 0.27	3.13 ± 0.15
Methyl hexanoate	<0.5	<0.5	<0.5	<0.5	<0.5
(Z)-Methyl geranate	3.31 ± 0.08	4.93 ± 0.06	3.63 ± 0.03	3.61 ± 0.11	2.65 ± 0.05
Isoamyl isobutyrate	<1.0	<1.0	<1.0	<1.0	1.38 ± 0.25
Methyl heptanoate	<2.0	<2.0	<2.0	<2.0	<2.0
Methyl decanoate	6.80 ± 0.52	3.48 ± 0.35	3.25 ± 0.26	4.42 ± 0.19	3.07 ± 0.24
(E)-Methyl geranate	65.64 ± 5.16	79.71 ± 1.37	57.21 ± 1.35	87.73 ± 4.54	67.50 ± 1.62
Methyl octanoate	<2.0	<2.0	<2.0	<2.0	<2.0
Methyl nonanoate	<3.0	<3.0	<3.0	<3.0	<3.0
Geranyl acetate	<3.0	<3.0	<3.0	<3.0	<3.0
Sum of esters	**75.75**	**91.38**	**67.36**	**98.92**	**77.73**
% of esters	**1.3**	**2.6**	**1.4**	**1.5**	**1.1**
β-Pinene	1384.54 ± 170.13	778.83 ± 46.80	904.48 ± 34.42	1138.58 ± 78.01	1101.61 ± 83.69
Myrcene	353.21 ± 32.95	398.93 ± 16.31	363.21 ± 18.07	466.83 ± 33.00	418.31 ± 20.25
Limonene	5.76 ± 0.5	6.35 ± 0.54	6.03 ± 0.29	8.40 ± 1.06	8.28 ± 0.53
(E)-β-Ocimene	61.53 ± 5.73	39.26 ± 1.13	38.10 ± 2.69	55.02 ± 4.50	50.44 ± 1.36
3-Carene	34.89 ± 2.23	27.44 ± 1.83	32.37 ± 2.03	40.64 ± 2.91	41.24 ± 1.01
α-Pinene	<1.0	<1.0	<1.0	<1.0	<1.0
Sum of monoterpens	**1839.93**	**1250.81**	**1344.19**	**1709.47**	**1619.88**
% of monoterpens	**32.1**	**35.2**	**27.7**	**25.8**	**22.6**
Terpinen-4-ol	<1.0	<1.0	<1.0	<1.0	<1.0
α-Terpineol	5.23 ± 0.63	4.00 ± 0.14	2.43 ± 0.47	3.34 ± 0.48	4.74 ± 0.43
(Z)-Geraniol (Nerol)	<2.0	<2.0	<2.0	<2.0	<2.0
Linalool	25.33 ± 1.05	33.98 ± 0.74	22.24 ± 0.71	46.35 ± 1.21	42.94 ± 0.46
Farnesol	25.94 ± 8.97	23.73 ± 1.38	75.97 ± 2.57	249.63 ± 6.10	472.46 ±12.96
β-Caryophyllene oxide	55.40 ± 9.57	37.89 ± 2.29	12.65 ± 0.48	26.98 ± 1.44	19.28 ± 1.39
Sum of alcohols	**111.90**	**99.60**	**113.29**	**326.30**	**539.42**
% of alcohols	**2.0**	**2.8**	**2.3**	**4.9**	**7.5**
(E)-β-Farnesene	611.99 ± 71.65	743.19 ± 10.85	1174.97 ± 21.24	1645.14 ± 62.96	1196.47 ± 32.28
β-Caryophyllene	771.26 ± 101.62	394.79 ± 7.34	598.57 ± 12.58	887.71 ± 27.36	941.03 ± 28.82
α-Humulene	2112.50 ± 249.88	897.23 ± 30.83	1448.47 ± 49.22	1833.86 ± 115.25	2722.25 ± 81.06

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Sum of sesquiterpens	**3495.75**	**2035.21**	**3222.01**	**4366.71**	**4859.75**
% of sesquiterpens	**61.1**	**57.2**	**66.5**	**65.9**	**67.8**
2-Decanone	14.59 ± 0.42	3.86 ± 0.63	8.13 ± 0.25	8.69 ± 0.58	3.44 ± 0.63
2-Undecanone	101.46 ± 4.97	41.36 ± 0.59	49.30 ± 0.47	59.11 ± 1.27	32.59 ± 1.25
2-Dodecanone	11.64 ± 0.2	4.38 ± 0.06	7.34 ± 0.11	8.94 ± 0.35	4.35 ± 0.09
2-Tridecanone	67.72 ± 7.36	29.39 ± 1.18	34.56 ± 1.19	45.03 ± 4.73	26.95 ± 2.52
β-Ionone	<2.0	<2.0	<2.0	<2.0	<2.0
α-Ionone	<2.0	<2.0	<2.0	<2.0	<2.0
2-Nonanone	6.37 ± 0.56	<2.0	2.27 ± 0.35	3.12 ± 0.22	1.72 ± 0.19
α-Irone	<2.0	<2.0	<2.0	<2.0	<2.0
Sum of carbonyls	**201.78**	**78.99**	**101.60**	**124.89**	**69.05**
% of carbonyls	**3.5**	**2.2**	**2.1**	**1.9**	**1.0**

mean value of six measurements ± standard deviation.

Our methodology allowed comparison of a profile of 31 hop oils forming basic groups as monoterpenes, sesquiterpenes, their oxidized forms or alcohols, esters, and aldehydes and ketones referred to in the text as carbonyls among Saaz varieties. Following known facts, the monoterpenes and sesquiterpenes represent the main constituents of studied hops [24,25]. Monoterpenes ranged from 1250 to 18400 mg/kg, which represents 22.6 to 35.2% of quantified compounds. The comparison of monoterpenes is given in Figure 1, the highest concentration of monoterpenes has Saaz, and the lowest S. Late. Particularly, the highest concentration of β-pinene (780–1385 mg/kg) followed by myrcene (353–466 mg/kg) was found there. The concentration of (E)-β-ocimene is about 40–60 mg/kg. Sesquiterpenes are the most abundant group ranging from about 2050 to 4860 mg/kg found in Saaz and S. Brilliant, respectively. It represents 57.2 to 67.8%. α-Humulene is the dominant (about 900–2720 mg/kg), followed by β-farnesene (about 610–1650 mg/kg) and β-caryophyllene (about 390–950 mg/kg). The other compounds represent a significantly smaller fraction, alcohols from 2.0 to 7.5% (represented especially by farnesol, linalool, and β-caryophyllene oxide), esters from 1.2 to 2.7%, and carbonyls from 1.0 to 3.5%. This distribution is given in Figure 1.

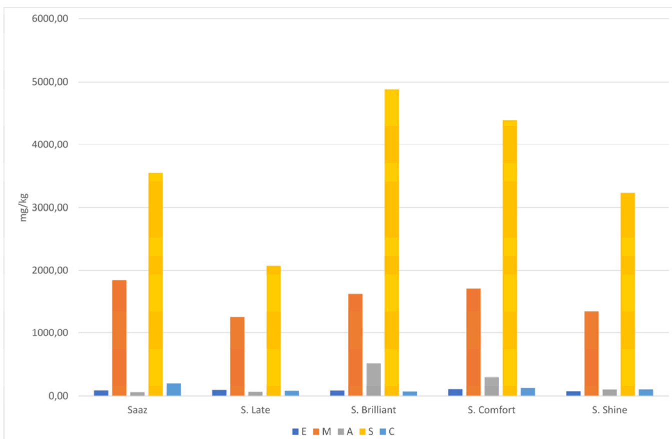

Figure 1. Profile of hop oil groups in Saaz varieties.

Visualization of samples in multivariate space is shown in Figure 2. Every variety formed its well-separated cluster. It closely correlates with the genetic origin of these varieties and the aroma of hops [4]. While S. Late and S. Brilliant have in their genotype the ancestor according to original Saaz, S. Comfort and S. Shine were bred using different varieties, such as Serebrianka and Sládek, respectively. Equally, the aroma of Saaz, S. Late, and S. Brilliant is similar (fine hoppy aroma with floral and herbal scent), however, S. Comfort and S. Shine have a sharper and more intense aroma with spicy and fruity, respectively, in the background.

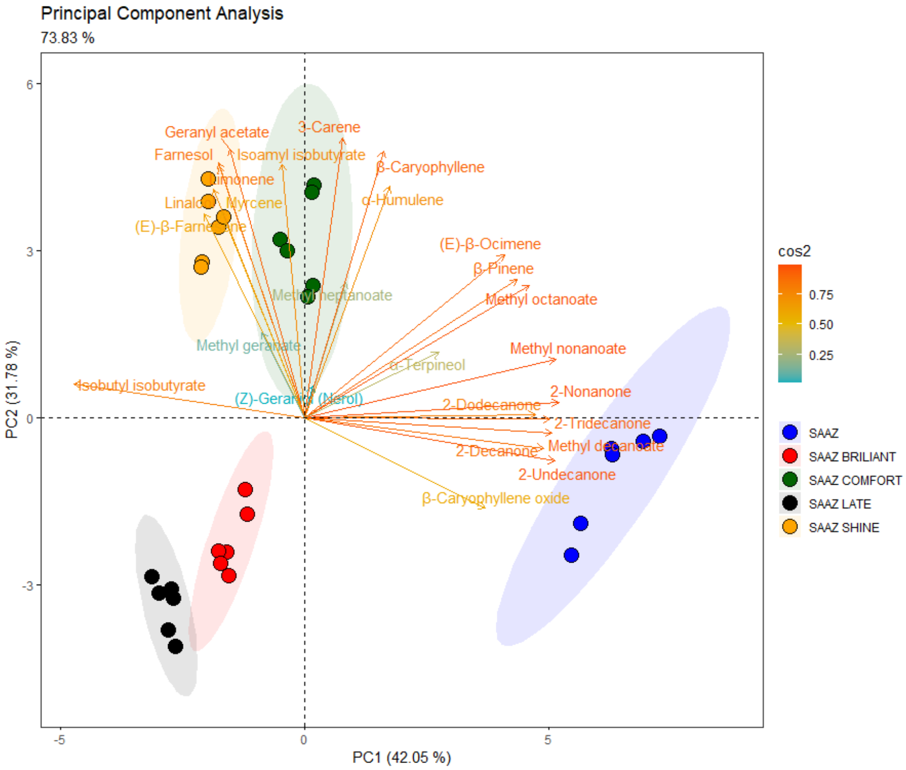

Figure 2. Principal component analysis of Saaz varieties and their hop oils content.

In general, the main profile of hop oils in the Saaz variety correlates with a study from 2012, where authors identified in Saaz variety 27 terpenoid metabolites, representing monoterpenes (56.1%), sesquiterpenes (34.9%), oxygenated monoterpenes (1.41%), and hemiterpenes (0.04%) [6]. Eyres who performed a comparison of tentatively odor-active compounds in Saaz, Target, Hallertauer Hersbrucker, and Cascade varieties identified 14-hydroxy-β-caryophyllene as a responsible compound of "woody and cedarwood", and further geraniol, linalool, and α-ionone. The Charm value of geraniol and linalool was the highest among the four studied hop varieties [5].

The chemical profiles of hop oils correspond well with sensory analysis discussed in previous study [4]. In general, Saaz varieties have a traditional fine aroma with a typical pure hop aroma pronounced by the herbal and floral aroma. Both, S. Comfort and S. Shine have sharper aromas with a pronounced spicy and fruity scent, respectively. They are more noticeable in comparison with the other Saaz varieties. S. Comfort and S. Shine also form close clusters in the PCA graph given in particular by a higher concentration of geranyl acetate, myrcene, limonene, ocimene derivates, linalool, farnesol, β-farnesene, β-caryophyllene.

The basic parameters of beers, given in Table 3, show that the beers are very similar. The values of the original extract, alcohol by weight and volume occur close to 12.5% (w/w), 4.0% (w/w), and 3.0% (v/v), respectively. This is reflected in very similar apparent and real extracts (about 3.0 and 5.0, resp.). Color and haze are also well-balanced among the brews.

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A slightly larger difference was noted in the final bitterness that is ranging from 36 to 41 BU which is caused in particular by a different proportion of α-acids homologs (discussed above). In practice, fluctuation in beer bitterness levels from brew to brew is in general significant and unwanted fact [26].

Further, beers were compared according to hop oils content by PCA (see Figure 3).

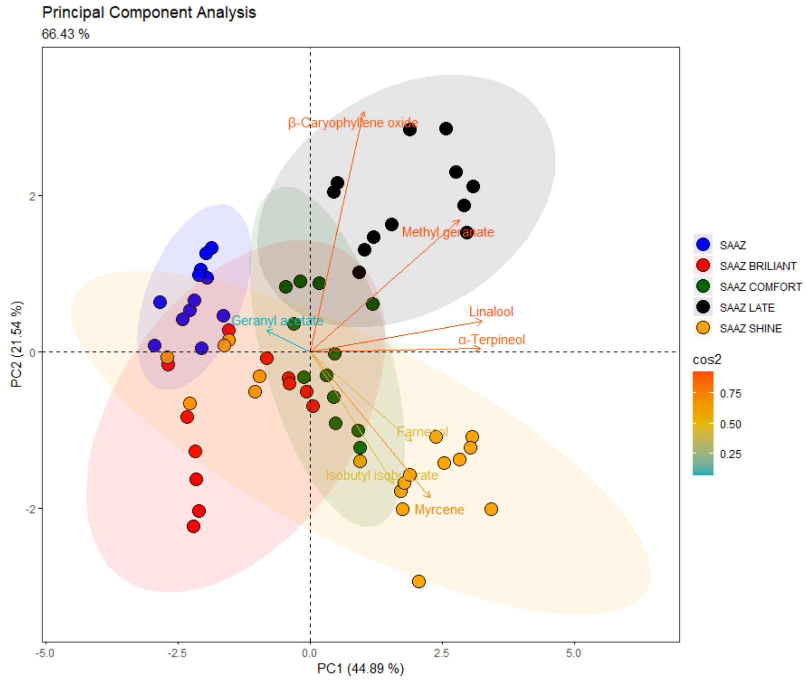

Figure 3. Principal component analysis of single-hopped beers hoped by relative Saaz varieties and their hop oils content.

Compared to Figure 2, single-hopped beers no longer form autonomous clusters which is caused by the biotransformation of hop oils during wort boiling and fermentation. The newly formed hop oils profile is shown in Figure 4 and Table 5.

Table 5. Hop oils profile in single-hopped beers hoped by relative Saaz varieties.

	Saaz	**S. Late**	**S. Brilliant**	**S. Comfort**	**S. Shine**
	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutyl isobutyrate	<1.00	1.34 ± 0.61	1.67 ± 0.61	1.06 ± 0.44	1.46 ± 0.52
Methyl hexanoate	<0.50	<0.50	<0.50	<0.50	<0.50
(Z)-Methyl geranate	8.48 ± 0.67	14.42 ± 2.32	8.49 ± 1.72	10.92 ± 1.07	10.48 ± 1.72
Isoamyl isobutyrate	<1.00	<1.00	<1.00	<1.00	<1.00
Methyl heptanoate	<0.50	<0.50	<0.50	<0.50	<0.50
Methyl decanoate	<0.50	<0.50	<0.50	<0.50	<0.50
(E)-Methyl geranate	<1.00	<1.00	<1.00	<1.00	<1.00
Methyl octanoate	<0.50	<0.50	<0.50	<0.50	<0.50
Methyl nonanoate	<0.50	<0.50	<0.50	<0.50	<0.50
Geranyl acetate	1.30 ± 0.38	1.71 ± 0.63	2.71 ± 1.46	2.01 ± 0.63	2.46 ± 1.98
Sum of esters	**9.78**	**17.47**	**12.87**	**13.99**	**14.54**
% of esters	**7.7**	**8.0**	**9.8**	**7.8**	**6.2**
β-Pinene	<0.5	<0.5	<0.5	<0.5	<0.5
Myrcene	2.71 ± 0.51	3.22 ± 0.85	3.37 ± 0.59	4.71 ± 1.17	5.05 ± 1.99

Limonene	<0.5	<0.5	<0.5	<0.5	<0.5
(E)-β-Ocimene	<1.00	<1.00	<1.00	<1.00	<1.00
3-Carene	1.59 ± 1.79	1.05 ± 1.61	1.98 ± 2.81	1.18 ± 1.38	1.05 ± 1.93
α-Pinene	<1.00	<1.00	<1.00	<1.00	<1.00
Sum of monoterpens	**4.30**	**4.27**	**5.35**	**5.89**	**6.10**
% of monoterpens	**3.4**	**1.9**	**4.1**	**3.3**	**2.6**
Terpinen-4-ol	0.46 ± 0.26	0.78 ± 0.35	0.61 ± 0.27	0.64 ± 0.23	0.55 ± 0.32
α-Terpineol	3.24 ± 0.25	5.12 ± 0.36	4.01 ± 0.78	4.41 ± 0.15	4.74 ± 0.84
(Z)-Geraniol (Nerol)	3.14 ± 0.38	2.04 ± 0.53	2.72 ± 0.16	1.90 ± 0.12	2.09 ± 0.25
Linalool	17.25 ± 1.46	32.11 ± 2.60	19.31 ± 3.43	28.82 ± 2.51	27.76 ± 7.50
Farnesol	**86.98 ± 30.04**	**155.34 ± 6.80**	**85.54 ± 31.24**	**122.00 ± 21.82**	**179.58 ± 10.00**
β-Caryophyllene oxide	1.49 ± 0.29	2.53 ± 0.22	0.64 ± 0.16	1.10 ± 0.13	0.80 ± 0.14
Sum of alcohol	**112.57**	**197.92**	**112.83**	**158.87**	**215.52**
% of alcohol	**88.5**	**90.1**	**86.1**	**88.6**	**91.3**
(E)-β-Farnesene	<2.00	<2.00	<2.00	<2.00	<2.00
β-Caryophyllene	<0.50	<0.50	<0.50	<0.50	<0.50
α-Humulene	0.50 ± 0.23	<0.50	<0.50	0.55 ± 0.30	<0.50
Sum of sesquiterpens	**0.50**	**0.00**	**0.00**	**0.55**	**0.00**
% of sesquiterpens	**0.4**	**0.0**	**0.0**	**0.3**	**0.0**
2-Decanone	<1.00	<1.00	<1.00	<1.00	<1.00
2-Undecanone	<1.00	<1.00	<1.00	<1.00	<1.00
2-Dodecanone	<1.00	<1.00	<1.00	<1.00	<1.00
2-Tridecanone	<1.00	<1.00	<1.00	<1.00	<1.00
β-Ionone	<0.50	<0.50	<0.50	<0.50	<0.50
α-Ionone	<0.50	<0.50	<0.50	<0.50	<0.50
2-Nonanone	<1.00	<1.00	<1.00	<1.00	<1.00
α-Irone	<0.50	<0.50	<0.50	<0.50	<0.50
Sum of carbonyls	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
% of carbonyls	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

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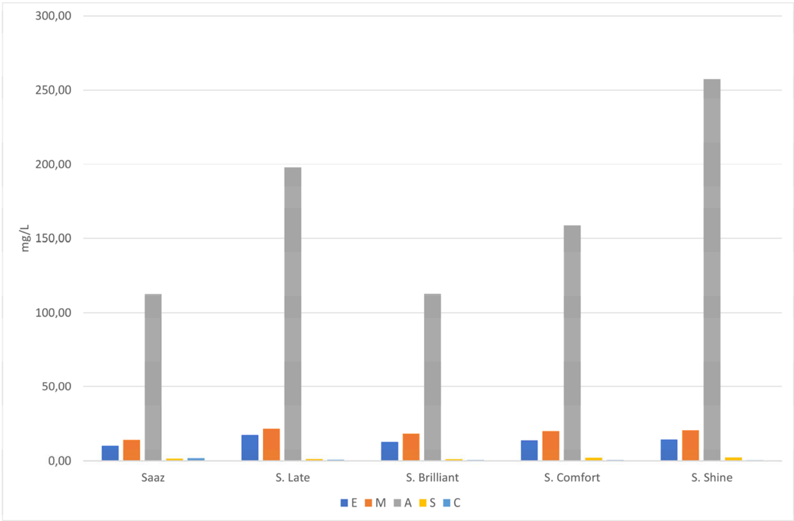

Figure 4. Profile of hop oil groups in single-hopped beers hopped by relative Saaz varieties.

While the main group fractions in hops are monoterpenes and sesquiterpenes, their oxidized forms are dominant in beer as published previously [24]. Due to the absence of polar functional groups, myrcene, α-humulene, and β-caryophyllene have very low solubility causing their evaporation during kettle hopping and also fermentation where they are washed out by carbon dioxide. Thus, in general, terpene hydrocarbons do not contribute to the hop aroma of beer except for dry-hopped beer [27,28]. However, their oxidized derivatives formed during wort boiling can have an impact on the beer aroma so-called "noble" or kettle hop aroma [29].

Oxidized forms of monoterpenes and sesquiterpenes represent 88.5–91.3%, which corresponds to a concentration of about 112–216 mg/l (see Table 5). The highest concentration of these alcohols was found in S. Shine. Farnesol contributes most to this concentration (86–180 mg/L), followed by linalool (17–32 mg/L), α-terpineol (3–5 mg/L), nerol (2–3 mg/L), and traces of terpin-4-ol (< 1 mg/L).

The thermochemical conversion of β-farnesene to farnesol was described previously A boiling model monitored the stereoisomeric changes of several terpene alcohols as a result of boiling was used. Farnesol was not detected in original hops but only in two samples after the thermochemical conversion [30]. The formation of farnesol was also mentioned in a review [24] and a recent study regarding the comparison of the sensory and chemical profile of kettle and kettle+dry single-hopped beers using S. Brilliant, S. Comfort, and S. Shine. Also in this case farnesol was a major sesquiterpene alcohol in kettle hop beer [10].

Very hypothetically, a production of some yeast strain could potentially be a source of farnesol in beer as described by Muramatsu who used squalene synthetase-deficient mutant Saccharomyces cerevisiae ATCC 64031 for farnesol production. However, optimal pH cultivation for the farnesol extracellular production was found from 7.0 to 8.0, further, an acidic medium with pH below 4.0 was optimal for the intracellular farnesol and its isomer nerolidol [31]. Naturally, a significant proportion of farnesol in beer may come directly from the hops, where was also detected. However significant concentrations of farnesol in hops were determined only in Saaz Brilliant, Saaz Comfort, and the largest in Saaz Shine (about 470 mg/kg). While in Saaz and Saaz Late its concentration was only about 25 mg/kg. Therefore, the possibility of the formation of farnesol from farnesene is quite probable.

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Linalool, α-terpineol, nerol, and terpin-4-ol are autoxidative products of myrcene during bowling and fermentation [24]. Moreover, monoterpene alcohols, namely linalool, geraniol, and also their isomers nerol and a-terpineol are produced in hops during hop ripening of hop cone, hop processing, and aerobic storage [32]. These terpene alcohols occur in hops as free volatiles and also esterified [33] and are bound to carbohydrates/glycosides [34]. During beer production, thermal, enzymatic, and acid-catalyzed cleavage of these compounds proceeds to cause aglycones' release, thus, increasing beer aroma [34].

The second group of hop oils, in order of concentration, consists of esters that represent 5 to 9% or 10–14 mg/L with the highest concentration of S. Late. The most abundant derivate among all varieties is Z-methyl geranate followed by geranyl acetate. The group of aldehydes and ketones is minimally represented, namely less than 0.5 mg/L.

The chemical results are in good agreement with sensorial analysis (see Table 6).

Table 6. Basic parameters of single-hopped beers.

	Saaz	S. Late	S. Brilliant	S. Comfort	S. Shine	Saaz
Fullness	3.0	2.7	2.9	3.0	3.0	3.0
Bitterness	2.1	2.2	1.8	1.9	1.9	2.1
Astringency	1.2	1.2	1.2	1.1	1.2	1.2
Sourness	1.4	1.5	1.3	1.5	1.6	1.4
Sweetness	1.5	1.5	1.5	1.6	1.6	1.5
Hoppy (overall)	2.0	2.1	2.3	2.3	2.1	2.0
Estery	1.3	1.2	1.2	1.2	1.4	1.3
Yeasty	1.0	1.3	1.2	1.0	1.0	1.0
Hop aroma (in detail)	hoppy, herbal, floral, spicy	hoppy, floral, fruity, woody, spicy	hoppy, fruity, herbal, floral, woody	hoppy, fruity, herbal, spicy	hoppy, fruity, floral, herbal, spicy	hoppy, herbal, floral, spicy

six-point scale (0 – none, 1 – very low intensity, 5 – very high intensity), data processing: trimmed mean value from 24 individual evaluations uncertainty was 0.5.

Beers have in general fine bitterness and a hoppy aroma with herbal and floral scents, they differ only in slight details. A hop aroma is formed by the synergy of both, major and minor hop oils, such as farnesol (floral, lemongrass, jasmine), linalool (floral), geraniol (floral, rose), α-terpineol (lilac, rose, woody), nerol (floral, lime, citrus), Z-methyl geranate (floral, herbal, fruity), geranyl acetate (fruity, floral), myrcene (herbal, resinous, green, spicy) [35]. Only beers hopped by S. Brilliant and S. Comfort have a slightly stronger hop aroma, however, these detailed differences in the sensory profile were not distinguished by the projective mapping.

These results are also in agreement with long-term results of hop evaluation where Saaz varieties are hardly recognizable to untrained assessors. Very similar aromas have Saaz and S. Brilliant, S. Comfort has a little higher aroma intensity and S. Shine has the most noticeable fruity scent with traces of citrusy of all [4]. These fine differences are almost not apparent when traditional kettle-hopped technology is used. On the other hand, slightly bigger differences will show after dry-hopping as was recently published [10].

4. Conclusions

These findings show that "new" varieties of the Saaz pedigree are well used for the production of the Pilsner type of lager without affecting the traditional sensory aroma of this widespread style. This is a particularly important insight from the climate change perspective. Moreover, new varieties have a higher yield and concentration of bitter acids and hop oils. Further, the high concentration of farnesol and linalool in beer correlated with a high concentration of farnesol and linalool in beer correlated with a high concentration of β-farnesene and myrcene in Saaz hops, respectively, significantly contributing to the typical fine hop aroma of Pilsner lager.

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Nomenclature

AEDA: Aroma Extract Dilution Analysis; BU, Bitterness Units; GC-O, Gass Chromatography with Olfactometry; GC-MS, Gass Chromatography with Mass Spectrometry; PCA, Principal Component Analysis.

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