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Phytochemical and sensory comparison of Czech and world flavour hop cultivars

In the new millennium, a lot of hop flavour cultivars with a unique “varietal aroma” have been bred and widely used for a dry hopping of beers. Seven flavour hop cultivars were also registered in the Czech Republic in recent years. Essential oils, depending on their concentrations, combinations and threshold levels, are responsible for diverse aroma and flavours of hop cultivars in dry cones and beers. In this study, we evaluated qualitative essential oils parameters and sensory aroma profiles in dry cones of 7 Czech and 23 world flavour hop cultivars by multivariate principal component analysis (PCA), which divides cultivars to quadrants. The Czech flavour cultivars Juno and Jupiter were placed in the first quadrant, influenced by higher geranyl acetate and methyl-4-8-decadienoate contents. The Kazbek, Ceres, Eris, Pluto and Saturn cultivars were grouped in the second quadrant, influenced by higher 2-MeBu-isobutyrate and geranyl isobutyrate contents, in essential oils analyses. However, in sensory aroma analyses, the Jupiter cultivar was placed in the first quadrant due to its woody aroma, whereas the Juno and Pluto cultivars were placed in the second quadrant due to their floral and grassy aromas. The Saturn cultivar was placed in the third quadrant due to a fruity aroma and the Kazbek, Ceres and Eris cultivars were grouped in the fourth quadrant due to their citrusy aroma. 7 Czech and 11 world flavour hop cultivars were also compared by sensory aroma analyses in dry hopping brewing tests. There was no correlation found between cultivar sensory aroma profiles in dry cones and beers, and differences and discrepancies in flavours for all of them. We proved that Czech hop flavour cultivars provided original sensory aroma profiles in beers and that they are useful for breweries in different beer styles.

Descriptors: flavour hop cultivars, essential oils compounds, sensory aroma profiles, principal component analysis (PCA), dry hopping, beer

1 Introduction

Traditional hop (*Humulus lupulus* L.) cultivars have been used in brewing industry for a long time. In the new millennium, a craft beer market has started an increasing demand for new hop cultivars with new aroma and flavours. Therefore, hop breeders have provided new hop cultivars every year in all hop growing regions. Nowadays, there are 333 hop cultivars in the IHGC hop variety list [1]. These cultivars fall into just two groups: These cultivars fall into just two categories: “aroma” and “bitter”. But some of the aroma hop cultivars contribute a characteristic “varietal aroma” of different flavours (citrusy, fruity, floral, etc.) to finished beer. These cultivars with an attractive, sensory-specific and unconventional aroma are defined as “flavour hops,” which are widely used for dry hopping of beers [2]. The first cultivar of flavour hops bred in

the Czech Republic has been Kazbek in 2008 [3]. In recent years, a new generation of Czech flavour hop cultivars has emerged: Juno, Ceres, Saturn, Jupiter, Eris, and Pluto were registered in 2022 and 2023 [4]. These cultivars have special aromas that are ideal for dry hopping of beer. They have been tested in brewing trials [5, 6, 7] and are currently used in large and small breweries. Even though their chemical characteristics and sensory parameters are known, the brewing industry cannot use them in a suitable, required and efficient way because a comparison to popular aroma intensive hop cultivars as Citra or Mosaic is missing.

It is known that the volatile compounds in hop essential oils add key aroma and flavour to the final beer product, depending on the hopping technology used during the brewing process. The composition of hop essential oils is highly diverse, over 450 volatile substances have been identified, and dried hop cones typically contain between 0.5–3% hop oils on average [8]. Hop essential oils generally consist of three chemical groups: hydrocarbons, oxygenated compounds and sulphur-containing compounds. These groups account for 60–80%, 20–40% and less than 1% of the total essential oils in hops, respectively. The quantity and composition of hop essential oils depend largely on the hop variety, genetic factors, the age of hop plant or rootstock, growing conditions, including soil, pH level, carbon and nitrogen content, moisture content and microbial mass. They also depend on climatic conditions, such as (temperature, humidity, sunshine hours), as well as the time of harvest [8, 9]. The most abundant compounds in the hydrocarbon fraction are the monoterpene myrcene and the sesquiterpenes β -caryophyllene,

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α -humulene, β -farnesene and selinenes [6]. The other two chemical groups, oxygenated and sulphur-containing compounds contribute more to the hop aroma and flavour of beers [2].

The group of oxygenated compounds includes a complex mixture of oxygenated sesquiterpenoids, alcohols, aldehydes, acids, ketones, epoxides, and esters [8]. The first major flavour compounds contributing to hop varietal flavours from are monoterpene alcohols. There is mainly linalool which has a floral, lavender-type flavour, geraniol which has a rose-like floral flavour and β -citronellol which has a lime-like citrus flavour [2, 10]. Nerol and α -terpineol support a fresh, fruity and citrus-like flavour [8]. Esters are the second most important flavour compounds. The main esters are isobutyl isobutyrate, isoamyl isobutyrate, and 2-methyl butyl isobutyrate. These isobutyric esters have fruity, green apple, grape, melon-like and apricot-like flavours [2,10]. Methyl esters, in particular, contribute to the hop aroma and flavour of beer due to their low threshold concentrations, such as ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, ethyl-2-methylpropanoate, ethyl-4-methylpentanoate, methyl-2-methylbutanoate, methyl geranate and geranyl acetate.

These esters have fruity (apple, plum and pear), citrusy and tropical fruit-like (pineapple and sweet) flavours, as well as green and floral aroma, but also waxy aroma [8]. However, some esters, such as ethyl octanoate and ethyl butyrate, bring “unpleasant”, “solvent-like” or “cheesy” flavours to beer [8].

The group of sulphur-containing compounds (thioesters, sulphides, and other sulphur-containing compounds) are the most interesting flavour compounds contributing to hop varietal flavours [11]. They are mostly sensory active substances with threshold levels in ng/L and can therefore easily affect the overall aroma of hops and beer [9]. The most important of them, such as dimethyl sulphide (DMS), dimethyl disulphide, dimethyl trisulfide, diethyl disulphide, 2-methyl-3-furanethiol, 2,3,5-trithiahexane, S-methylthiomethyl 2-methylpropanethioate, and S-methylthiomethyl 2-methylbutanethioate, impart unpleasant flavour characteristics of cooked vegetables, cabbage, leek, onion or garlic to beers [8]. 3-methyl-2-butene-1-thiol (3MBT) imparts a light-struck “skunky” off-flavour to beer [2]. But hop breeders and brewmasters have mainly been focused on other “fruity” volatile thiols, such

Table 1 Essential oils composition in dry cones of seven Czech flavour hop cultivars. Relative content expressed in percentage

Cultivar	Kazbek		Ceres		Juno		Jupiter		Pluto		Saturn		Eris	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
Total oils (g/100 g)	1.24	1.09	1.17	1.24	0.66	0.94	0.82	0.84	0.87	1.11	0.93	1.62	1.34	1.03
Myrcene	25.2	31.7	26.3	29.1	38.7	37.4	44.8	41.5	42.2	31.3	42.4	27.5	23.4	22.1
Beta-pinene	1.69	0.86	1.42	0.85	2.54	1.81	2.00	1.36	1.69	1.32	1.93	1.24	1.40	1.05
Cis-ocimene	1.98	0.69	0.78	0.29	1.40	0.54	2.09	1.95	0.22	0.06	0.29	0.14	3.31	1.56
Caryophyllene	14.0	13.4	12.1	14.6	10.5	12.6	11.3	15.6	7.14	9.50	10.4	13.0	11.8	12.9
Farnesene	0.75	1.01	0.06	0.17	0.39	0.14	0.57	0.37	0.30	0.08	0.22	0.28	0.14	0.12
Humulene	16.6	21.2	25.4	32.6	3.21	1.24	4.37	2.00	15.6	24.4	22.4	26.3	19.8	24.2
Selinenes	4.77	3.78	1.92	2.11	7.00	8.55	9.57	11.8	2.02	1.92	2.12	1.24	4.60	4.85
Linalool	1.19	0.65	1.12	1.39	1.59	1.39	1.58	0.83	1.05	0.68	1.27	0.66	0.88	0.61
Geraniol	0.38	0.24	0.06	0.04	0.40	0.27	0.25	0.18	0.51	0.39	0.28	0.22	0.20	0.09
2-undecanone	0.18	0.22	0.25	0.12	0.36	0.27	0.48	0.30	0.35	0.22	0.27	0.17	0.27	0.22
Isobutyl isobutyrate	0.41	0.15	0.07	0.03	0.47	0.54	0.25	0.10	1.86	0.94	0.71	0.37	1.60	1.40
2-MeBu-isobutyrate	2.24	1.41	0.36	0.14	1.80	0.98	0.76	0.65	4.02	3.07	2.85	1.57	3.89	2.93
Methyl octanoate	0.31	0.22	0.31	0.18	1.92	1.40	1.40	0.63	0.33	0.20	0.14	0.13	0.05	0.04
Methyl-4-decenoate	1.44	1.35	1.72	1.41	1.98	1.51	2.16	1.85	1.70	1.43	1.31	1.40	0.17	0.09
Methyl-4-8-decadienoate	0.85	0.61	1.20	0.96	2.35	2.08	1.48	0.76	1.36	0.97	0.51	0.46	0.13	0.05
Methyl geranate	0.92	0.33	1.21	0.87	0.44	0.40	1.43	1.22	0.39	0.24	0.48	0.51	0.13	0.12
Geranyl acetate	3.09	1.66	1.20	1.12	5.30	5.95	2.85	1.87	3.97	3.50	2.75	1.69	2.08	1.44
Geranyl propionate	0.98	0.88	0.78	0.47	0.64	0.61	1.55	1.12	2.85	1.46	0.97	1.15	1.11	0.75
Geranyl isobutyrate	2.52	1.76	1.64	1.31	5.52	5.81	1.77	2.60	5.26	5.11	1.67	1.92	3.40	3.07
Caryophyllene oxide	0.69	0.23	0.35	0.28	0.79	1.87	0.53	0.38	1.73	0.90	0.47	0.91	0.99	1.78
Humulene epoxide II	0.72	0.37	0.87	0.83	0.06	0.13	0.13	0.16	3.58	2.71	1.10	2.16	1.65	3.45

were selected from the 63 measured compounds [9] for statistical analyses.

2.3 Beer preparation

Brewing tests were conducted with 18 selected flavour hop cultivars intended for dry hopping: seven Czech (Kazbek, Jupiter, Juno, Saturn, Pluto, Ceres, Eris), seven from the United States (Simcoe, Centennial, Cascade, Ekuanot, Sabro, Citra, Mosaic), two from New Zealand (Motueka, Nelson Sauvin), one Australian (Galaxy) and one from German (Mandarina Bavaria). The test were carried out in a pilot brewery (BREWTEC s.r.o., Litvinov, CR) of the Hop Research Institute Co.Ltd. in Zatec [7]. Top-fermented beers with dry hopping were produced for this study in 2022. Wort was prepared from Pilsen (Maltery Klusacek, Kounice, CR), Pale Ale and Wheat Pale malts (Weyermann, Bamberg, Germany) using the infusion mashing to 12 – 12.4 OG. The 90 minutes brew was hopped 3 times (1/3 dosage at 5, 40 and 80 minutes) with 3g/l of Vital hop pellets to a target beer bitterness of 40 IBU. After a hot trub separation in a whirlpool, the wort was cooled down to the fermentation temperature of 20 °C. The primary fermentation was carried out in cylindroconical tanks (Pacovske strojirny a.s., Pacov, CR), using Fermentis SafAle US-05 yeast (Fermentis, Marcq-En-Baroeul, France). After one week, the green beer was cooled down to a temperature of 11 °C and transferred to 5 or 10 liter KEG casks. Dry-hopping by the tested hop cultivars was performed immediately using a static procedure with a uniform dose of 3 g/L of loose hop pellets in kegs for 11 days. Beers were cooled down to a temperature of 2 °C after 3 days. The beers were filtered using a cellulose plate filter before being bottled using a machine filler with double evacuation and pressurisation of the bottles with carbon dioxide.

2.4 Sensory analyses of hops and beers

The dry hop cone rub method has been used for sensory aroma profile evaluation of the Czech hops cultivars during the crop years 2016 – 2021 by the Breeding department of the Hop Research Institute Co., Ltd. in Zatec [4]. The intensity of the seven individual flavours (herbal, fruity, citrusy, floral, spicy, grassy and woody) of the hop aroma were evaluated using an ascending scale ranging from 0 to 5. Sensory aroma profiles of all the world commercial cultivars were obtained from the cultivar data sheets on merchant's and breeder's websites [12, 13, 14, 15, 16]. Different sensory aroma profiles were reduced to the seven main flavours and standardized to a range from 0 to 5.

A skilled tasting panel (three women and eight men) of beer judges,

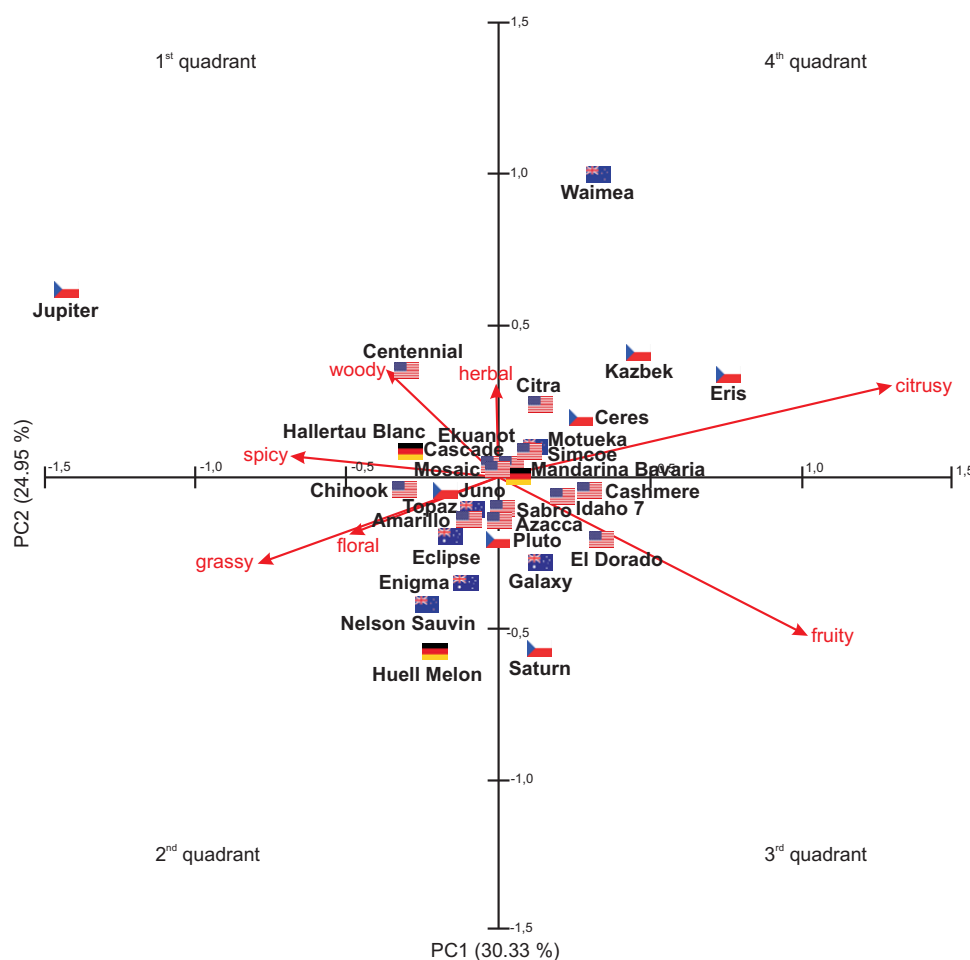


Fig. 2 Principal Component Analysis bi-plot of 30 world hop flavour cultivars based on sensory scores of 7 main aromas in dry cones

all of whom had professional certificates, was recruited for the beer sensory analysis. The same sensory aroma profile evaluation was used for an intensity of seven individual hop flavours in beer. Beers were evaluated twice in three series of six blind sample, in random order. The results obtained were averaged.

2.5 Statistical analyses

As the analytical data of the individual essential oils compounds had a wide range of values, they were first standardised using min-max normalisation to a range of values from 0 to 5. The statistical similarity of individual hop samples was performed on the basis of the Euclidian distance matrix, which was used for the multivariate principal component analysis (PCA) in DARwin v. 5.0.155 (Dissimilarity Analysis and Representation for Windows, <http://darwin.cirad.fr/darwin>). The same statistical analyses were used for hop sensory aroma profile evaluation of cones and beers. A Mantel test for PCA distance matrices comparison was run in PASSaGE v.2.0.11.6 (<http://www.passagesoftware.net>) [17]. Bi-plot graphs were visualised using CorelDRAW 11 (Alludo, Ottawa, ON, Canada). Spider plot graphs of hop cultivar sensory profiles in cones and beers were visualised using Microsoft Excel (Microsoft Office 2016, Microsoft Corporation, Redmond, WA, USA).

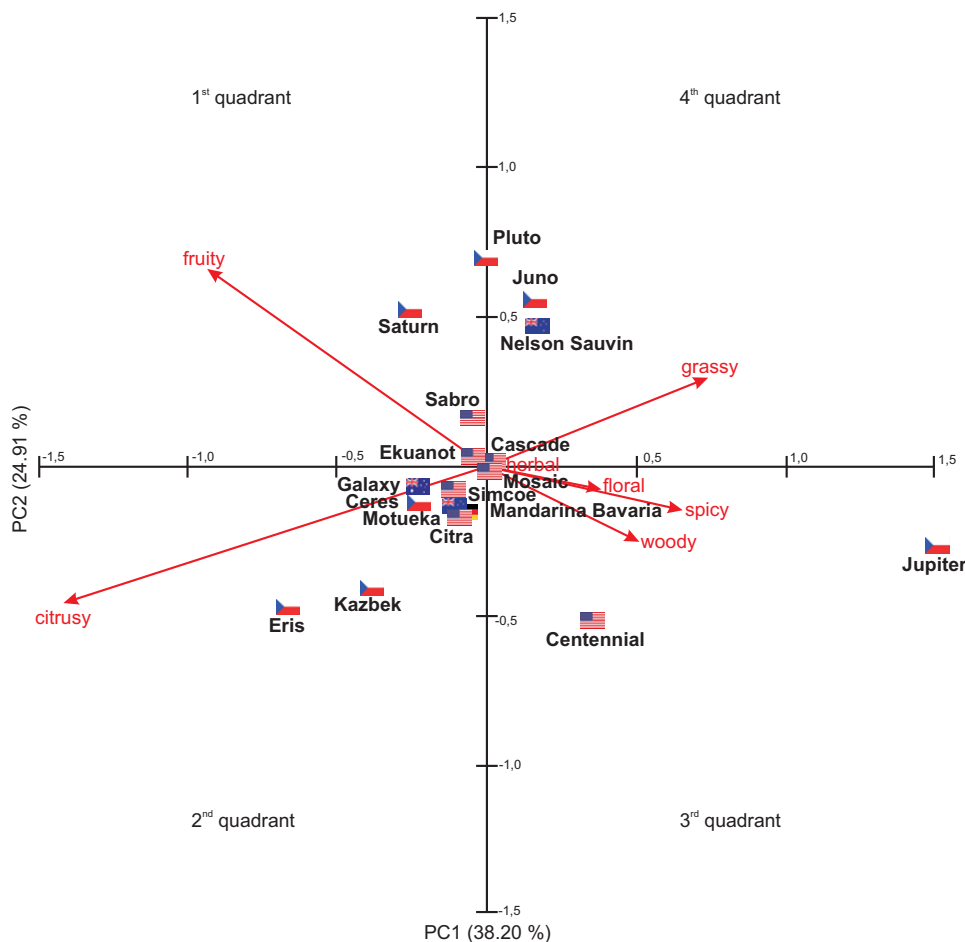


Fig. 3 Principal Component Analysis bi-plot of 18 selected hop flavour cultivars based on sensory scores of 7 main aromas in dry cones

3 Results and discussion

3.1 Phytochemical and sensory evaluation of the hop cone samples

The phytochemical characterisation of 30 studied hop cultivars comprised the quantification of the total hop essential oils content and 21 individual compounds. Table 1 summarises the content of total essential oils and their composition in seven Czech flavour hop cultivars over two crops. The measured values were within the range of cultivar parameters [4] and were similar to previous results [6]. Differences were observed between crop samples of the same hop cultivars, because the quantity and composition of hop essential oils depend on the crop year, the time of harvest and other factors [8, 9].

A multivariate approach employing the principal component analysis (PCA) was carried out in order to compare Czech flavour hop cultivars with the studied set of world flavour hop cultivars. Figure 1 shows PCA bi-plot of all 30 hop samples in the first two dimensions, when PC1 explained 21.62 % and PC2 15.39 % of total variance. The values were significantly lower than in previous results ranging from 60 % to 95 % together [18, 19, 20, 21]. This could be due to the greater diversity of cultivars in our study. Juno cultivar with the highest contents of geranyl acetate and methyl-4-8-decadienoate was clustered in the first quadrant together with Jupiter cultivar.

The highest content of myrcene influenced its clustering. Isobutylisobutyrate, geranyl propionate, methyl octanoate and cis-ocimene were next relevant compounds for this quadrant, where Australian cultivars Topaz and Enigma and the US cultivar Citra were placed. The other Czech flavour cultivars Kazbek, Ceres, Saturn, Eris and Pluto were clustered in the second quadrant together with New Zealand cultivar Nelson Sauvin. This quadrant was influenced by the contents of 2-MeBu-isobutyrate, β -pinene, caryophyllene, humulene, geranyl isobutyrate and total oils. The US cultivars Azacca, Amarillo, Cashmere, Ekuanot, El Dorado, Chinook, Cascade, Simcoe and Sabro were clustered in the third quadrant. The contents of linalool, 2-undecanone, methyl-4-decenoate, caryophyllene oxide and humulene epoxide II influenced this quadrant. Whereby the US cultivars Idaho 7, Centennial and Mosaic, the German cultivars Huell Melon, Mandarin Bavaria and Hallertau Blanc, the New Zealand cultivars Waimea and Motueka, and the Australian cultivars Galaxy and Eclipse were clustered in the fourth quadrant. This quadrant was influenced by the contents of geraniol, methyl geranate, farnesene and selinenes. Previous studies have used a range from 4 main terpenes [20] to

61 essential oils compounds [21] for PCAs of world hop cultivars [18, 19]. Identical compounds were clustered in correlation with previous results. This enabled us to compare the analyses for some of the cultivars. New Zealand cultivars Waimea and Motueka were placed similarly in the study by Killeen et al. [20], but cultivar Nelson Sauvin was clustered together with Waimea cultivar due to more used essential oils compounds in the study by Purdy et al. [21]. Australian cultivars Galaxy, Topaz and Enigma were placed similarly as the US cultivar Cascade in the previous study based on 24 essential oils compounds [18].

A multivariate PCA was also used for sensory aroma profiles evaluation to compare results with essential oils PCA results. There was no correlation with $r = 0.05234$ [17]. Figure 2 shows PCA bi-plot of all 30 hop samples in the first two dimensions, when PC1 explained 30.33 % and PC2 24.95 % of total variance. Of the Czech flavour hop cultivars, the Jupiter cultivar was placed in the first quadrant due to its high woody aroma. Spicy and herbal aromas also influenced this quadrant. The Juno cultivar was placed in the second quadrant. The US cultivar Chinook as well as the Australian cultivar Topaz had the most similar sensory profiles. Grassy and floral aromas influenced this quadrant. The Pluto cultivar was clustered on the border of the second and the third quadrant close to the US Cultivar Azzaca. The Saturn cultivar was placed in the third quadrant due to its dominant fruity aroma. Due to their dominant citrusy aroma the Kazbek, Ceres and Eris cultivars were clustered in the

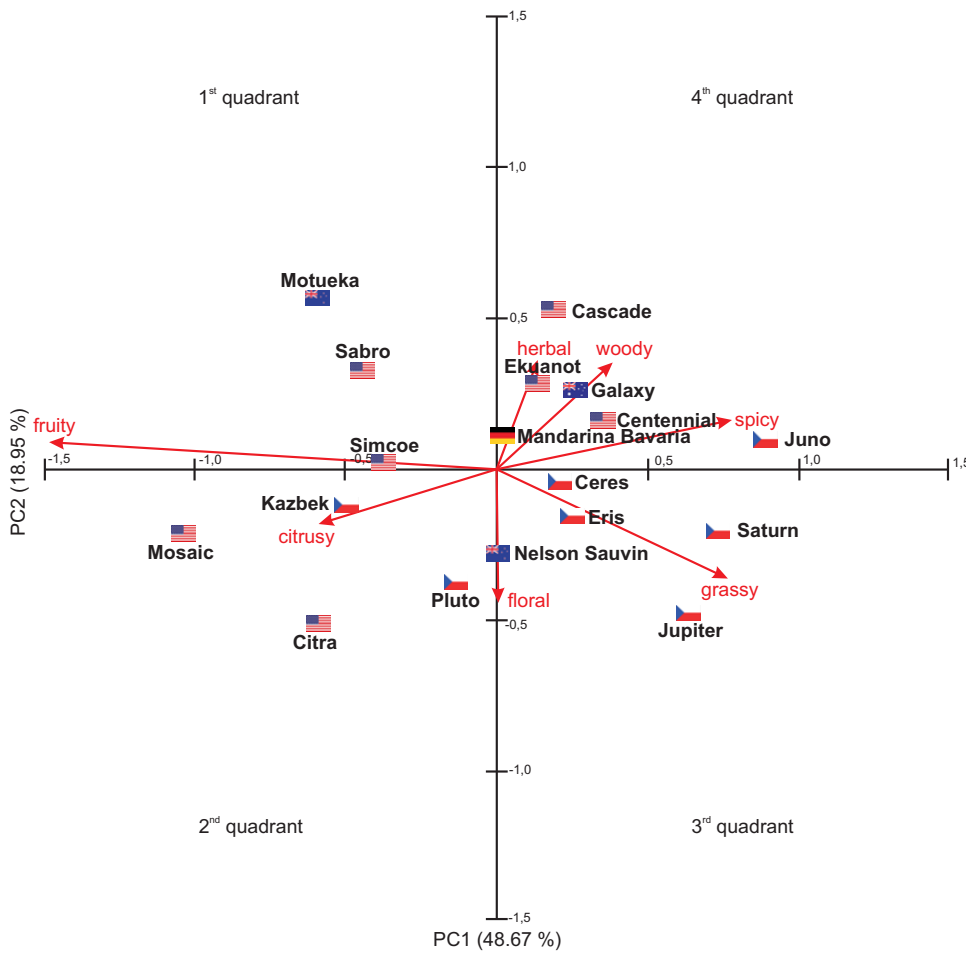


Fig. 4 Principal Component Analysis bi-plot of 18 selected hop flavour cultivars based on sensory scores of 7 main flavours in beer

fourth quadrant. Between four [11] and 12 different flavours [12] were used for sensory aroma profiles by the hop breeders and merchants. This general sensory hop-profile information for hop cultivars can differ from that of individual cultivar hop samples [22]. However, the PCA clustering results were similar to those of previous studies for some identical cultivars [10, 22, 23].

3.2 Sensory evaluation of Czech and world flavour hop cultivars in beer

Seven Czech and eleven world flavour hop cultivars were selected from 30 studied hop samples for brewing tests. Therefore, a multivariate PCA was recalculated for sensory aroma profiles of dry cones. Figure 3 shows PCA bi-plot of 18 flavour hop samples in the first two dimensions, when PC1 explained 38.2% and PC2 24.91 % of total variance. There were only small differences and quadrants' orientation (1st changed to 3rd, 2nd to 4th, 3rd to 1st and 4th to 2nd) for PCAs of 18 dissected flavour hop cultivars and 30 studied hop flavour cultivars (fig. 2).

A multivariate PCA was used for sensory aroma profiles evaluation in beer to com-

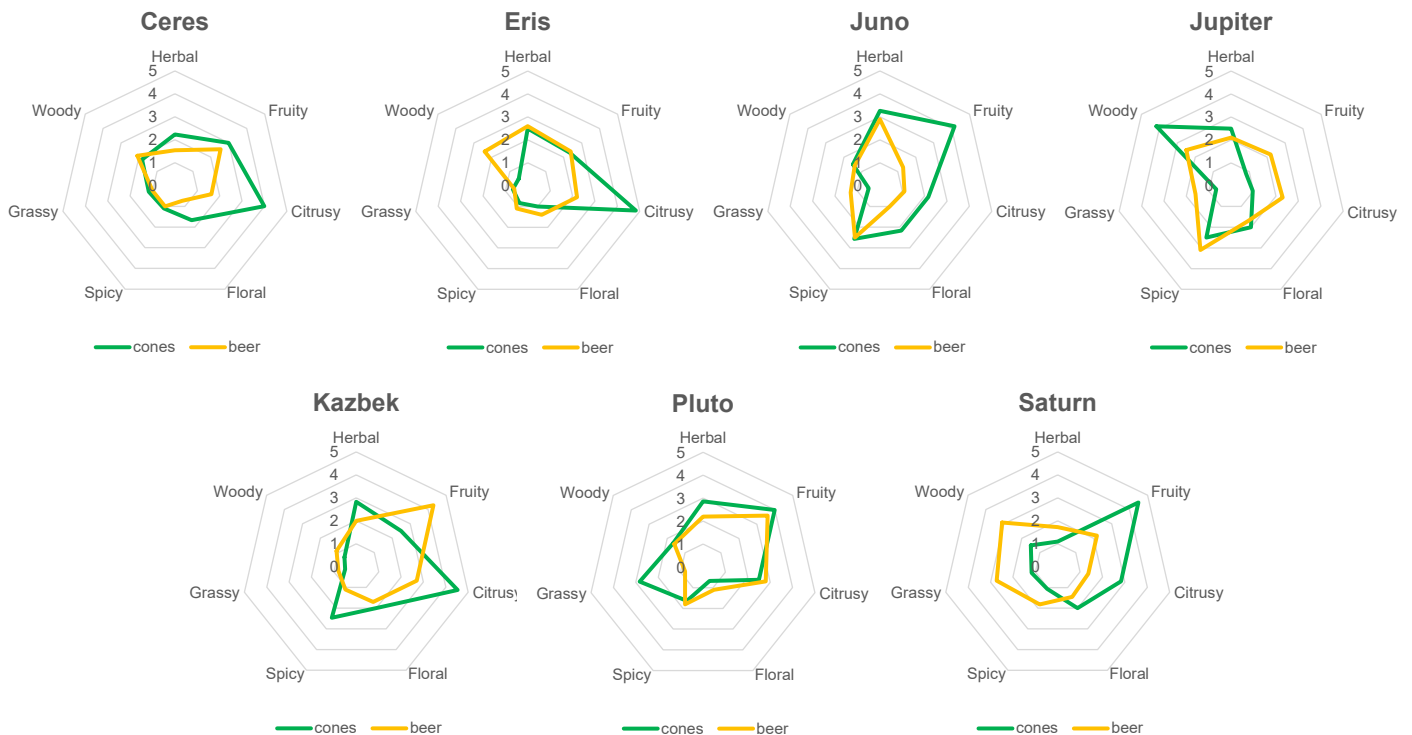


Fig. 5 Spider plot sensory profiles for 7 Czech hop flavour hop cultivars in dry cones and beer

pare results with dry cones PCA results. There was no correlation with negative $r = -0.16276$ [17]. Figure 4 shows PCA bi-plot of 18 single dry hopped beer samples in the first two dimensions, when PC1 explained 48.67 % and PC2 18.95 % of total variance. This variance was similar to previous results [22, 24]. It is known that the cultivar sensory aroma profiles of dry cones and beers depend on the recipes used, and discrepancies were found between the two [22]. From the Czech hop flavour cultivars, the Kazbek cultivar kept citrusy flavour (fig. 4), and the intensities of fruity flavour was enhanced and spicy flavour was suppressed in the beer sample (fig. 5), similarly to previous results [9]. The Ceres cultivar lost the intensities of citrusy, floral and herbal flavours in the beer sample (fig. 5), similarly to previous results [5, 6, 7]. The Juno cultivar lost also the intensities of citrusy, fruity and floral flavours, but the intensity of grassy flavour was enhanced in the beer sample (fig. 5), which moved this cultivar to a more spicy flavour in PCA (fig. 4). However, previous studies showed similar results [5] of keeping the citrusy flavour intensity [6]. These differences could be due to different crop samples [8, 9]. The Jupiter cultivar lost the intensity of its woody flavour, while the intensities of citrusy, fruity, grassy and spice flavours were more intense in the beer sample (fig. 5), which moved this cultivar within PCA (fig. 4). Previous studies showed similar sensory aroma profiles in beer [5, 6]. The Pluto cultivar lost only the intensity of grassy flavour in the beer sample (fig. 5), similarly to previous results [6, 7]. The Saturn cultivar produced the most distinct beer (fig. 5), where the intensity of fruity, citrusy and floral flavours was suppressed, and the intensity of grassy, woody, herbal and spicy flavours was enhanced. These findings moved this cultivar to the opposite position in the PCA (fig. 4). Similar results were found in previous studies [7], which were also influenced by the dry hopping dose [6]. The high intensity of the citrusy flavour was lost in the Eris cultivar, while the intensity of woody flavour was enhanced in the beer sample (fig. 5), which also moved this cultivar within the PCA (fig. 4). Previous studies have shown that similar sensory aroma profiles can be found in beer [5, 6]. All of the Czech hop flavour cultivars had the original sensory aroma profiles in beer and we didn't find any similar flavour cultivars from elsewhere in the world (fig. 4). They are well suited for the dry hopping of lagers and ales to enlarge their beer flavours. Previous studies [22, 24] placed the US cultivars Citra, Sabro, Simcoe, Centennial and Ekuanot in a similar way in PCAs. In contrast, the Australian cultivar Galaxy was close to the US cultivar Citra (fig. 4) [22].

4 Conclusion/Summary

Based on the evaluation of qualitative phytochemical essential oils parameters as well as sensory aroma flavour profiles in dry cones and dry hopped beers, we found that the Czech hop flavour cultivars provide original sensory aroma profiles in beers, fully expanding the range of world flavour cultivars. We confirmed in this study that compounds in hop essential oils contribute a multisensory experience of flavours in dry hopped beers but due to cross-modal interactions of individual compounds and metabolic biotransformation in beer we cannot exactly define and predict a cultivar sensory aroma profile in dry cones or beers. Therefore, sensory analyses of dry hop cones and beers are still very important for a cultivar sensory aroma profile definition and can be supplemented by phytochemical cultivar characteristics. The results obtained have

practical applications for breweries, such as the use of Czech flavour hop cultivars. This will increase the popularity of these hops for use in different styles of beer and boost future production. They are all useful for a kettle hopping of alcoholic and non-alcoholic pale lagers and ales. But their main potential is in the dry hopping. Use a single hop for dry hopping India Pale Lagers (IPLs), bitter hops for kettle hopping India Pale Ales (IPAs), or a combination of two or three hops for kettle and dry hopping of New England India Pale Ales (NEIPAs).

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