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Kazbek – The First Czech Aroma "Flavor Hops" Variety: Characteristics and Utilization

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Abstract

The world market of craft beer, especially dry hopped beers, has been constantly growing in the last few years. That is why new varieties of hops are still being bred. This article gives the genetic, agronomic, chemotaxonomic and brewing characteristics of Kazbek, the first "flavor hops" variety bred in the Czech Republic. Kazbek genetically belongs to a group of bitter American hops resulting from Brewers Gold variety, but the alpha acids content is relatively low, 5.0–8.0% by weight. The content of a homologous series of geranyl esters (acetate – propionate – isobutyrate) in an amount 2–4% of total essential oils (1–1.5 g/100g) is considered to be the originator of the specific, mainly citrus-like aroma of Kazbek. Moreover, the essential oil fraction contains about 25 sulfurous substances, predominantly thioesters. In the pilot scale brewing tests, the quality of Kazbek hops was proved both in kettle and dry hopping of Pilsner lagers. The overall sensory impression of kettle hopped lagers was comparable to Saaz hops, the differences were in the character of hop-derived flavor and bitterness. The best results of dry hopped beers were achieved for a 2.5 g/l hop dose and a contact time of 3 days.

Keywords: hop (*Humulus lupulus L.*), hop breeding, DNA, hop oils, beer, aroma, sensory analysis

1 Introduction

Under the term "aromatic hops", most brewers understand traditional European varieties such as Saaz, Hallertauer Mittelfrüh, Tetnang, Fuggle or derived hops like Sládek, Premiant, Spalter Select, Hall. Tradition, Liberty, Willamette, Sterling, Cascade, etc. Over the past 10 years, the term "aromatic hops" has gained a much wider meaning in the form of "flavor hops". These hops are characterized by an attractive, sensory-specific and unconventional aroma, which is widely used in beer because they are widely used for dry hopping. Depending on the time of the addition of hops, the type of beer and the composition of the beer matrix, these beers acquire an entirely unique hop flavor. Sensorially important are the compounds that evaporate without utilization from the kettle during wort boiling. This also applies to sulfuric substances, the amount of which in oils is about 1%. However, they are mostly sensory active substances with very low thresholds of perception, which can easily, mostly negatively, but also positively affect the aroma of hops and beer. Unpleasant scents have the character of cooked vegetables, cabbage or onion, pleasant aroma smells include those of tropical fruit or black currant. Volatile polyfunctional thiols 3-sulfanyl-4methylpentan-1-ol (3S4MP) and 3-sulfanyl-4-methylpentyl acetate (3S4MPA) are responsible for a unique aroma of beers with the character of exotic fruit, grapefruit and white wines. They were found out not only their synergistic interaction, but 3S4MP also increased the intensity of terpenic alcohols linalool and geraniol (Takoi et al., 2009). The 4-sulphanyl-4-methylpentan-2-one (4MMP) was identified as the

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origin of the extremely strong fruit aroma of blackcurrant in the US and Australian varieties Cascade, Simcoe, Summit, Apollo and Topaz. whose sensory threshold in beer is 1.5 ng/L (Kishimoto et al., 2006).

The varieties used for late and dry hopping are not classified in the traditional way as aromatic – bitter – high alpha because the alpha acid content is not essential for these hops. To the brewing process is usually added at a time when the bitterness of beers is nearly unchanged due to isomerization of alpha acids. Many flavor hops, however, are characterized by high alpha acids content. This is due to the fact that a large amount of bitter substances is often associated with high content of hop oils, up to about 3 g/100 g (Forster and Gahr, 2013).

Extraction of volatile substances during dry hopping is greatly facilitated by ethanol, which is already present at this stage of beer production. Dry hopping is the domain of small breweries in particular, but industrial breweries are already starting to use this technology to produce special beers (Verstl, 2018). Breeding of "flavor hops" is a great challenge for hop breeders. The first hops of this category, such as Citra, Amarillo or Simcoe, were bred in the United States in response to the demands of a rapidly growing craft breweries segment. Perhaps the greatest popularity has been acquired in recent years by the American Citra variety (Probasco et al., 2010). Beer from small breweries, whose US number exceeded 6600 in 2018 (Verstl, 2018a), finds more and more enthusiasts. Wide assortment of beers and their sensory diversity are the main attributes that distinguish this segment of the brewing industry from mass production. The unprecedented rise in the popularity of small breweries has caused a great request for aromatic hops the past several years. Other countries such as Australia (Galaxy variety), New Zealand (Nelson Sauvin) Germany (Polaris, Hallertau Blanc, Mandarin Bavaria, Huell Melon) (Kammhuber, 2013) were gradually added to the cultivation of flavor hops.

The first variety of flavour hops breed in the Czech Republic is Kazbek. The article summarizes its agronomic, genetic and chemotaxonomic characteristics. It also presents the results of aging tests and pilot brewing tests for both kettle and dry hopping application.

2 Material and methods

2.1 Genetic characteristics of the variety

The SSR method (Hadonou et al., 2004; Jakse et al., 2002), STS and EST-SSR marker systems were used for molecular-genetic analyses, (Patzak et al., 2007; Patzak and Matoušek 2011). A typical PCR reaction was carried out under the following amplification conditions: 2 min at 94 °C, 35 cycles (30 s at 94 °C, 60 s at 54 °C, 90 s at 72 °C); 10 min at 72°C. PCR was performed on a TGradient thermocycler (Biometra, Goettingen, FRG). The amplified products were distinguished by vertical electrophoresis in a 5% denaturing polyacrylamide gel and visualized by silver staining. For products, their presence or absence in individual samples was recorded based on molecular sizes of 20 bp DNA Marker (Bio-Rad, Hercules, CA, USA). he results of the amplified polymorphism were processed by a hierarchical cluster analysis based on the Jaccard coefficient of similarity (NTSYS-pc v.2.01, Exeter software, New York, NY, USA) by the method of Unweighted Pair Group Method with Arithmetic means (UPGMA,) in DARwin v. 5.0.155. The resulting dendrogram was visualized using Geneious Pro 4.8.2 programme (Biomatters Ltd., Auckland, New Zealand).

2.2 Chemotaxonomy

The characteristic contents and composition of alpha and beta acids, prenylflavonoids of Kazbek hops were determined over several years by analysis of samples taken from regionalization experiments of the Hop Research Institute, variety experiments of UKZUZ (Central Control and Testing Institute for Agriculture) and production hop yards in several localities within hop growing regions. The content and composition of hop resins were determined by EBC 7.5 method (EBC Analysis Committee, 2010). EBC 7.7 method determined the content and composition of alpha acids, beta acids, xanthohumol (XN) and desmethylxanthohumol (DMX). Analyses were performed on a SHIMADZU LC 20A liquid chromatograph. Isolation of hop oils was performed by distillation method (EBC Method 7.12). The content of essential oils was determined as the weight of oil extracted during the 90-minute atmospheric boiling of 100 g of hops. In the obtained oils, the total composition and the composition of sulfur compounds were determined by gas chromatography in combination with a mass detector (GC/MS) and a specific flame photometric detector (GC/FPD. The total composition analysis was performed on a DB 5 MS column (30 mx 0.25 mm x 0.50 µm) with a temperature program in the range of 60 °C to 250 °C on the THERMO-FOCUS gas chromatograph in conjunction with the DSQ II mass detector (Thermo Scientific). GC column was held at 60 °C for the first 5 min run time, followed by a ramp of 1.7 °C/min to 170 °C, 2.0 °C/min to 225 °C and 25 °C/min to 250 °C, where it was held for 5 minutes. The semiquantitative assessment of the composition of the essential oils was expressed in relative percentages as the percentage of the integrated area of the component to the total integrated area of all components of the essential oil. The identification of the components of the

essential oils was carried out by mass spectra and retention times of the analytical standards or with the help of library spectra.

Composition of sulfur compounds was analyzed on a Rxi-5Sil-MS column (30 mx 0.25 mm x 0.50 µm) temperature programmed in the range of 60 °C to 300 °C on a SHIMADZU 2010 Plus gas chromatograph combined with an FPD flame photometric detector -2010 Plus. The GC oven temperature was programmed as follows: 60 °C for 1 min, 3.0 °C/min ramp until 170 °C, then to 220 at 5.0 °C/min and to 300 °C at 25 °C/min. Column was held isothermally at 300 °C for 5 minutes. Identification of sulfuric substances has only been carried out on several components for which analytical standards are available. The whole analysis can be considered as a varietal "fingerprint".

The hop storage index HSI was measured on a Shimadzu UV-1601 UV-VIS spectrophoto-meter using the EBC 7.13 method. The content of total polyphenols was determined spectrophotometrically from hot-water hops extract according to the modified EBC method 8.12.

2.3 Storage stability

The storage stability of the variety reflects the loss of alpha acid content after 6 months of storage hops at room temperature and under air access (Nickerson and Likens, 1979). For the Kazbek variety, it was established within the framework of a long-term comprehensive comparative trial in which aging dynamics was evaluated under identical conditions for other Czech hop varieties for 12 months. The experiment was established during September 2017, when all alpha and beta acids were measured by EBC 7.7 and the EBC 7.13 hop index was determined. The analytical evaluation was repeated after 6 and 12 months of storage in March and September 2018.

2.4 Brewing trials

The brewing trials with the Kazbek hop products have been carried out on several pilot scale levels in recent years, using both raw and granulated hops dosed both in kettle and dry hopping in the course of beer maturation.

2.5 Kettle hopping

As an example of beer brewing tests, the results are presented of three-year brewing trials (2 hL) of pale lager beer with T90 pellets carried out at the Research Institute of Brewing and Malting (RIBM). Comparative brews were hopped by Saaz pellets. Hop pellets from the current harvest came from the production of the Hop Research Institute in Žatec (HRI).

Beer was made in accordance with the regulations of the PGI Czech beer (Commission, 2008). The preparation of wort of all-malt brews of the 12% pale lager was made by a two-mash decoction procedure. Hopping in three doses was 30% at the beginning, 50% after 30 minutes and 20% of hops 15 minutes before the end of 90-minute atmospheric wort boiling. The brews were hopped on the bitterness value of about 30 IBUs. After hot break removal, cooling down to the fermentation temperature of 10 °C and aeration at a dissolved oxygen content of 8 ± 0.5 mg/l the wort was pitched with yeast strain no. RIBM-95. The main fermentation was carried out in cylindroconical tanks (CCT) at the maximum temperature of 12 °C ± 0.1 °C. The maturing time in the lager tank was 30 days at 1–2 °C. The beer was filtered, bottled and pasteurized at 20 PU. Beer analyses were performed according to the EBC (EBC Analysis Committee, 2010). The essential oils in beer were determined by the GC/MS method developed at RIBM (Mikyška et al., 2018). Sensory analysis of beer was carried out by a trained sensory panel of RIBM using descriptive methods and triangle test (EBC Analysis Committee, 2010). The determination of the time profile of the decrease in bitterness and the bitterness character was carried out in accordance with the procedure developed by RIBM (Mikyška and Čejka, 2013).

2.6 Dry hopping

The influence of the hops dose and the contact time in the dry hopping on the intensity of the aroma and the overall impression after drinking was tested on a classic Czech lager beer. Kazbek hops were added to finished, unfiltered beer in lager containers at the dose of 1.0, 2.5 and 4.0 g/l. The containers were placed in an air-conditioned room at +2 °C. At this temperature they were left for 3 and 12 days. A total of 6 experimental variants were evaluated in a simple ranking test by a panel of 11 evaluators who rated the intensity of the aroma and the overall impression after drinking.

3 Results and discussion

3.1 Origin and genetic characteristics

A large share of the genetic basis of the Kazbek variety belongs to the Northern Brewer variety. From the first crossing in 1969, after the open pollination, the Bor variety emerged (Anonymous, 2012). From the subsequent crossbreeding of the Bor variety and the male hops originated in Russia, the Kazbek was selected in 1984 (named after the highest mountain of the North Caucasus) and registered by the Central Institute for Supervising and Testing in Agriculture of the Czech Republic in 2008 (Anonymous, 2012).

The use of molecular genetic methods allows accurate and reliable identification of the hop variety and, at the same time, makes it possible to evaluate the genetic variability and similarity of hop varieties using hierarchical cluster analysis. In the resulting dendrogram, genotypes genetically nearest to each other are grouped to clusters. These methods were used in the genetic analysis of the variety Kazbek together with other Czech and world hop varieties (Figure 2). Kazbek genetically belongs to a group of American bitter hops clustered together with the Brewers Gold, Galena and Columbus varieties.

3.2 Agronomic properties

The plant has a very large habitus of cylindrical shape. Because of the huge habitat it is necessary to cultivate the plant in min. spacing 300 x 114 cm. Bine is reddish-green in color and 9–11 mm in diameter. Hop cones are elongated in shape. The tips of the bracteoles are diverted from the cone (Figure 1). The average weight of 100 cones is in the range of 20 to 27 g. Kazbek is a late variety with a vegetation time in the range of 134–141 days. It is tolerant to primary and secondary infections of downy mildew (Pseudoperonospora humuli) and powdery mildew (Sphaerotheca humuli). The yield of the variety is 2.1 to 3.0 tons of dried hops/ha.

3.3 Chemotaxonomy

The chemotaxonomic characteristics of the Kazbek variety based on the analysis of hop resins, hop oils and polyphenols are shown in Table 1. Local and yearly variability of the content and composition of alpha acids, beta acids, the prenylflavonoids xanthohumol and desmethylxanthohumol (DMX) between 2014 and 2018 is evident from data in Table 2.

The content of alpha acids is usually in the range of 5.0–8.0%, the beta acid content in the range of 4.0–6.0%. The alpha/beta ratio is generally greater than 1.00. Cohumulone and colupulone ratios of 35–40% and 55–60% rel. are very high and predestined by genetic origin. Thanks to these parameters, the Kazbek variety can be easily identified among other Czech varieties (Krofta and Patzak 2011). However, as a result of vintage weather conditions, the alpha and beta acids or co-analogue ratios may be outside of the boundaries in both positive and negative terms, as documented in Table 2. In particular, the high temperatures in July, usually associated with a lack of precipitation, significantly reduce the alpha acid content, especially for some varieties (Kučera and Krofta, 2009). Fortunately, Kazbek belongs to the less sensitive varieties (Krofta et al., 2017). The contents of total polyphenols and prenylflavonoids do not deviate from ordinary values for most hybrid varieties.

Figure 1 *Appearance of ripe cones of the Kazbek variety*

The amount of essential oils is usually in the range of 1.0–1.5 g/100 g. The results of the analysis of Kazbek hop oils by gas chromatography are shown in Figure 3. The majority of the essential oil is myrcene, similar to other varieties. In the case of dry hopping, it is considered as an

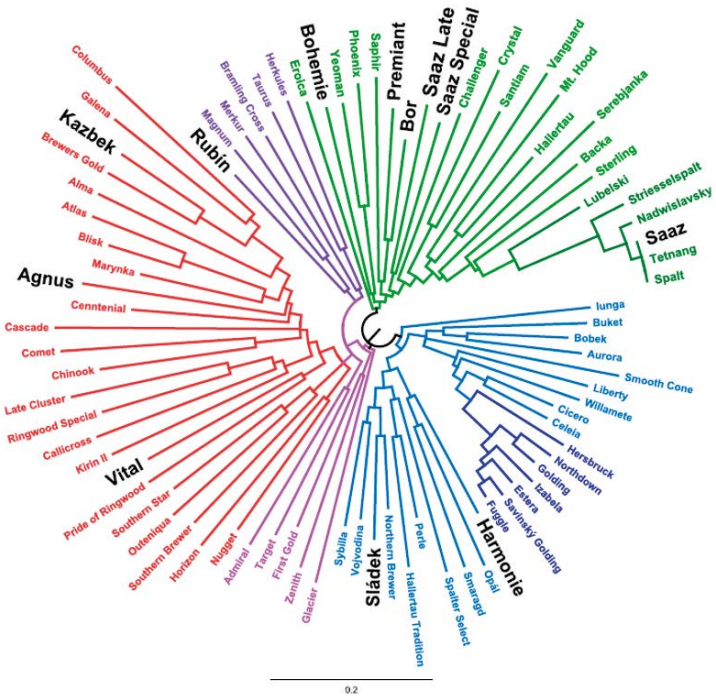

Figure 2 *Dendrogram of the genetic distances of 85 world hop varieties based on 238 polymorphic molecular markers.* Green – *hops of the European origin of the Saaz group*, blue – *hops of the European origin of the Fuggle group*, red – *hops of the American origin*, purple – *hops of mixed origin*, black – *Czech registered varieties.*

important component of the "floral" character of the beer aroma with a threshold of 13 µg/l in water (Van Opstaele et al., 2012).

The hybrid origin of the variety is confirmed by the presence of esters such as isobutyl isobutyrate, 2-methylbutylpropanoate, 2-methylbutylisobutyrate and 3-methylbutylisobutyrate.

For example, these esters are absent in essential oils of Saaz, but they are present in the Saaz Late variety resulting from multiple crosses of Saaz (Mikyška et al., 2013). The composition of the oils is also interesting, with an above-average content of cis-ocimene (more than 0.50% rel.) and a relatively low content of geraniol (0.10–0.15%). Linalool content around 0.50% does not deviate from normal values. However, the concentrations of both terpene alcohols can change due to yeast biotransformation during fermentation (King and Dickinson, 2003). Monoterpenic alcohols linalool and geraniol are associated with the "floral" odor of hops and beer (Nickerson and Van Engel, 1992). The amount of β-farnesene, α-selinene and β-selinene is negligible, unlike humulene, which is another major component (20–40%) of Kazbek hop oils. Sesquiterpenes are evaporated on a large scale during wort boiling. However, humulene and caryophyllene are partly found in the hops in the form of epoxides, which can pass up to the finished beer, especially in dry hopping (Yang et al., 1993). Only a few components of hops can be found in beer at concentrations exceeding their sensorial thresholds (linalool, geraniol, humuladienon, geranyl acetate) (Kishimoto et al., 2006). Also, the amount of the homologous series of methyl ketones is very small. For ex-

Table 1 *Chemotaxonomic characteristics of the Kazbek variety*

HOP RESINS	RANGE
total resins (% w/w)	17–22
alpha acids (% w/w)	5.0–8.0
beta acids (% w/w)	4.0–6.0
cohumulone (% rel.)	35–40
colupulone (% rel.)	57–62
POLYPHENOLS	
total polyphenols (% w/w)	3.5–4.5
xanthohumol	0.30–0.45
desmethylxanthohumol	0.10–0.20
HOP OILS	
Total oils (g/100 g)	*1.0–2.0*
isobutylisobutyrate (% rel.)	0.15–0.30
myrcene (% rel.)	35–50
2-methylbutylisobutyrate (% rel.)	1.00–1.50
limonene (% rel.)	0.15–0.25
linalool (% rel.)	0.30–0.50
geraniol (% rel.)	0.10–0.15
methylgeranate (% rel.)	0.15–0.25
geranylacetate (% rel.)	0.80–1.25
geranylpropionate (% rel.)	0.50–0.80
geranylisobutyrate (% rel.)	0.90–1.50
β-caryophyllene (% rel.)	8–13
α-humulene (% rel.)	20–40
β-farnesene (% rel.)	< 1.0
α- a β-selinenenes (% rel.)	1.0–3.0

Table 2 *Local and year variability of alpha acids, beta acids, xanthohumol and DMX of the Kazbek variety*

Year	Locality	alpha acids (% w/w)	beta acids (% w/w)	cohumulone (% rel.)	colupulone (% rel.)	xanthohumol (% w/w)	DMX (% w/w)
2014	1.	5.44	5.86	39.8	62.3	0.39	0.13
	2	4.32	4.56	36.6	59.2	0.24	0.11
	3	5.16	5.33	38.5	61.4	0.29	0.12
2015	1.	7.42	4.60	34.4	59.1	0.32	0.16
	2	3.97	3.90	34.9	57.2	0.26	0.09
	3	4.58	3.78	34.6	58.2	0.28	0.10
2016	1.	5.91	5.54	37.6	62.6	0.33	0.12
	2	5.32	5.98	37.9	61.3	0.37	0.09
	3	6.93	5.51	37.7	62.0	0.35	0.09
2017	1.	7.28	5.86	35.6	60.9	0.34	0.17
	2	4.60	4.25	35.5	58.9	0.29	0.09
	3	6.49	5.58	36.3	60.4	0.34	0.16
2018	1.	5.69	4.96	34.4	58.8	0.29	0.12
	2	5.20	5.21	34.3	56.7	0.32	0.13
	3	4.56	4.42	34.8	56.4	0.28	0.09

ample, the 2-undecanone content of 0.20% rel., which is usually abundant in most other varieties, is very small (Krofta, 2003). However, Kazbek hops contains several unique components, a homologous series of geranyl esters, geranyl acetate, geranyl propionate and geranyl isobutyrate (Figure 4). Their content is relatively high, about 2–4% rel. During fermentation, geraniol can escape into the beer by hydrolysis due to the enzyme activity of yeast (Takoi, 2010). Due to the significant presence of geraniol esters, Kazbek clearly differs from most of commercial hop varieties. Only the variety Cascade contains geraniol esters in a comparable amount (Sharp, 2014).

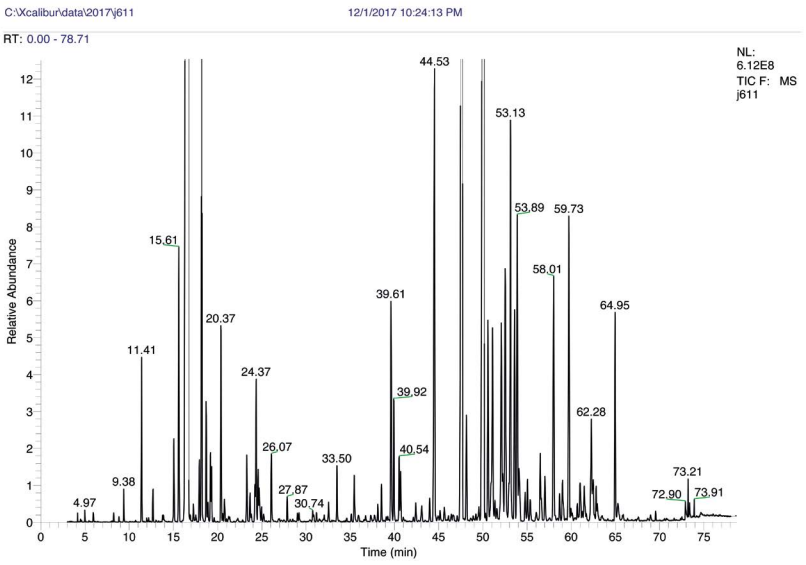

Figure 3 *GC chromatogram of hop oils of the Kazbek variety, isolation of essential oils by distillation method, column DB5, 30 m x 0,25 mm x 0,50 µ m, carrier gas helium, 60 kPa, split injection 1:50, (16,5 min, –myrcene; 44,4 min, - geranylacetate; 47,8 min, – β- caryophyllene, 50,1min, – α–humulene, 52–53 min, – α–+ β- selinens)*

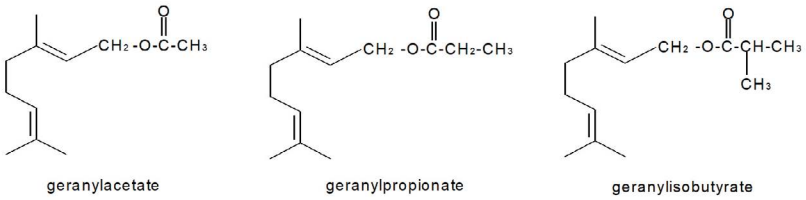

Figure 4 *A homologous series of geraniol esters found in hop oils of the Kazbek variety*

The total number of major sulfuric substances detected in hops oils is between 20 and 30. However, with a larger detector resolution, a number of other minor sulfur compounds, for example glycosidically bound thiols, can be found (Gros, 2011). Figure 5 shows the chromatograms of the major sulfuric substances in hop oils of the Kazbek and Saaz Czech varieties, where the second variety is being listed as comparative. The major constituents of the sulfur components of hop oils are methylthioesters, S-methylthiohexanoate (elution time 14:06 min) and S-methylthioisovalerate (7:77 min). Their sensory properties with the character of cooked vegetables are evaluated negatively (Peppard, 1981). A similar sensory profile is also characterized by dimethyltrisulfide (8:87 min), which, however, occurs only in trace amounts in the essential oils of the tested varieties. Other sulfuric substances such as 3-sulfanylhexanol (15:46 min) and 3-sulfanylhexyl acetate (21:39 min) were not detected at all. In the case of 4-MMP, co-elution with S-methylthioisovalerate (7:79 min) were observed on the chromatographic column. Separation of the two substances will require a column with different stationary phase.

It can be reasonably assumed that, in combination with other ingredients, geraniol esters are the carriers of specific fragrance of Kazbek hops, which has been officially classified by commercial firms as "flavor hops" (Der Barth Bericht, 2018), which includes the world varieties Citra, Amarillo, Cascade, Galaxy, Mandarina Bavaria, etc. The sommelier panel characterized the fragrance of Kazbek as "tangerine, mint, melon, coriander, grapefruit". The hop flavors are widely characterized by spider charts, which in a simpler design describe the five basic hop flavors such as herbal-fruity-spicy-floral-citrus (Whittock and Kotoulis, 2011). The application of this approach to the Kazbek variety (Figure 6) documents the distinctive citrus character of the aroma.

3.4 Storage stability

During the storage experiment of Czech hop varieties after the 2017 harvest, a reduction of alpha acid content in Kazbek hops from 5.91% to 4.52% was found after 6 months, which in relative terms represents a decrease of less than 24% rel. According to Nickerson and Likens (1979), the loss of alpha acid between 20 and 40% rel. represents a fair storage stability. The good storage stability of the Kazbek variety was also confirmed by the losses of alpha acid found under analogous conditions in other years (27% – 2014/2015; 40% – 2015/2016 a 39% – 2016/2017). A reliable indica-

tor of the aging dynamics of hops is hop storage index (HSI). Its value in fresh hops ranges from 0.25 to 0.30 (Cocuzza, 2013). During the storage and processing of hops on products its value irreversibly rises. The aging dynamics of the Kazbek and other Czech hop varieties in the form of leaf hops, expressed as a rate of increase in HSI over time, is shown in Figure 7. According to this indicator, Kazbek along with the Saaz Special, Sladek, Agnus, Vital, Gaia and Boomerang varieties belongs to the group of fast-aging hops. The aging rate of of Bor, Premiant and Saaz hops is considerably slower. The aging rate of hops significantly slows down by storage in air-conditioned warehouses at temperatures up to +5 °C. In the case of pellets, it is further improved by packaging into barrier bags under an inert atmosphere (Mikyška and Krofta, 2012). The results of the aging test of the Kazbek T90 pellets under different storage conditions have shown that the quality of the hops is stable in anaerobic and air-conditioned environment for a minimum of 12 months. HSI storage indices are below 0.40 and are therefore acceptable to most breweries.

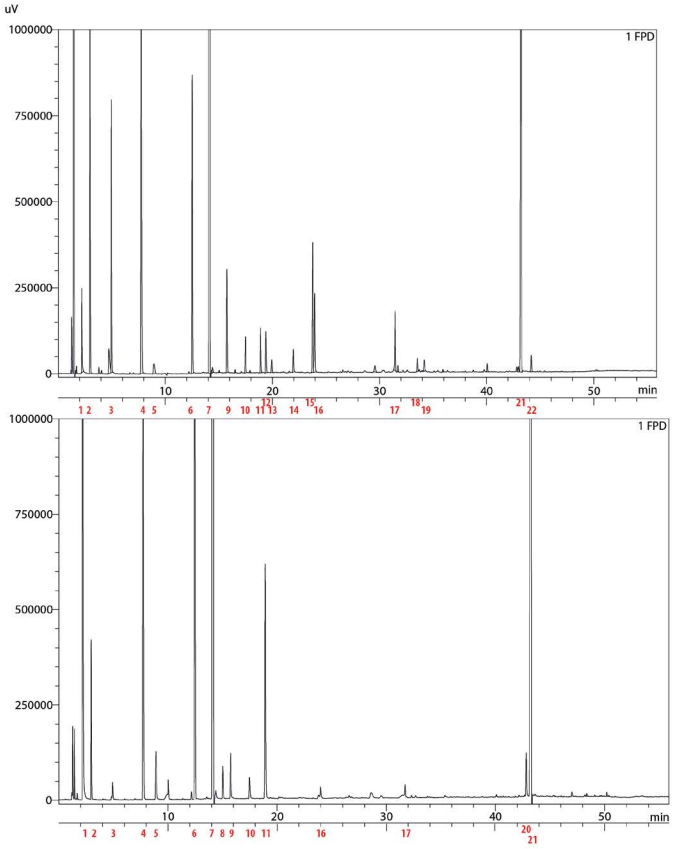

Figure 5 *Fingerprint of sulfuric substances in hop oils of Kazbek (top) and Saaz hops (GC / FPD, Rxi-5Sil-MS, 30 m x 0.25 mm x 0.50 µ m, temperature program 60 °C to 300 °C)*

3.5 Brewing tests

Kettle hopping: Brewing experiments were carried out in three consecutive years on materials from the current harvest. The values of the basic chemical analysis of beers document the balancing of the brews in terms of attenuation, bitter substances, color and foam stability (Table 3). The profile of essential oils in beer was partly different. The average content of terpenic alcohols linalool, cis-geraniol and farnesol was higher in beers hopped by Kazbek variety compared to beers hopped by Saaz. while the content of β-farnesen, α-humulen, α-terpineol and β-caryophyllene was higher in Saaz-hopped beer (Table 4). This is consistent with the contents of the mentioned substances in the hops used.

However, contrary to expectations, no substantial differences in geraniol concentrations were found in beers. The theoretical assumption of geraniol release from geranylesters of the Kazbek variety during fermentation has not been confirmed in this case (Takoi et al., 2017).

The sensory quality of all beers was at a good level, the score of overall sensory impression of beers brewed with Saaz and Kazbek pellets was comparable in all years (Saaz/Kazbek: 3.4/3.8; 4.4/4.6; 3.3/3.0), so there was no significant difference in the overall impression between the Saaz and

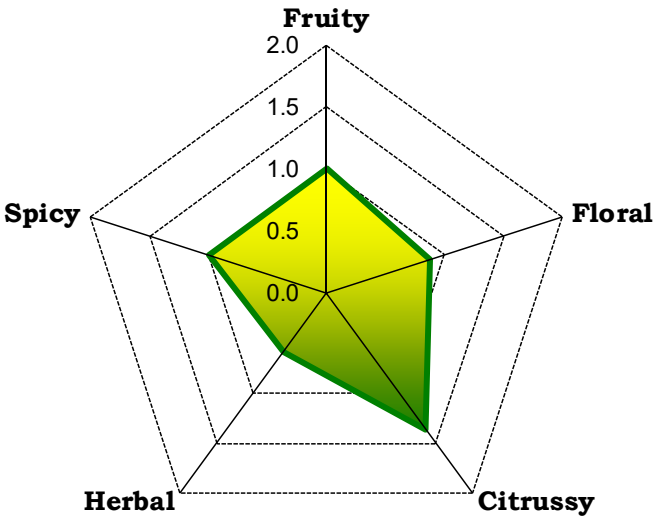

Figure 6 *Sensory profile of hop oils of the Kazbek variety*

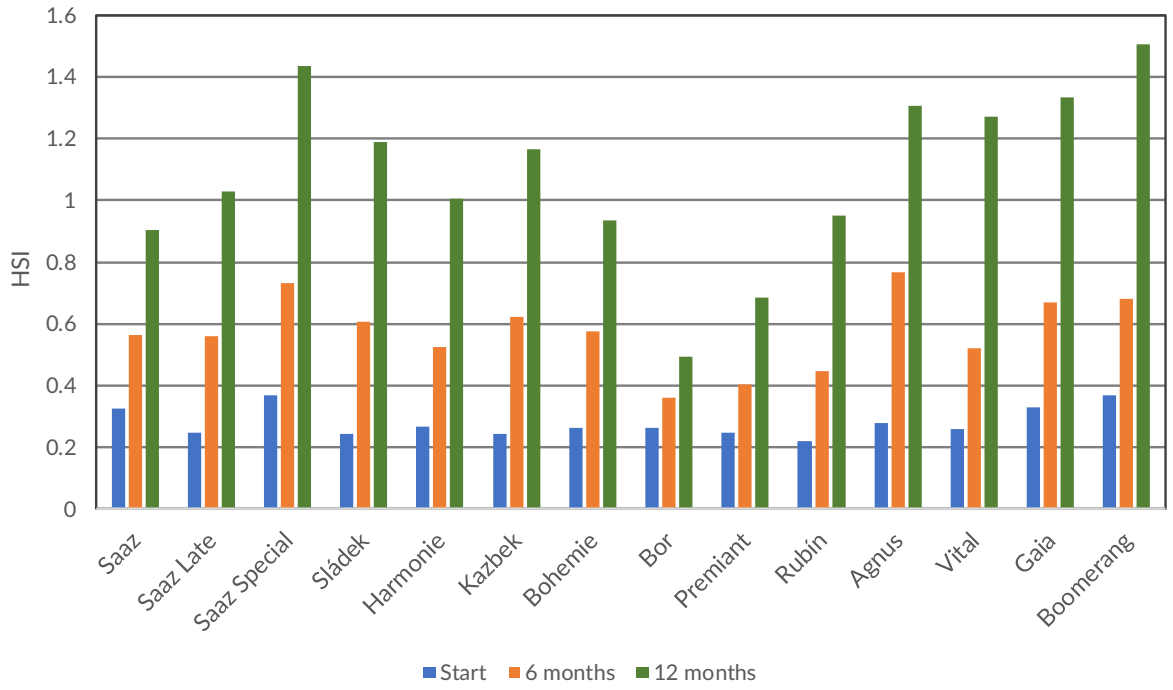

Figure 7 *Hop storage index (HSI) of raw hops of Czech varieties after storage in the dark and at room temperature for 6 and 12 months (harvest 2017/2018)*

Kazbek hops at the three-year average. In the sensory profile of beer there is a striking higher intensity of the hop flavor in the beers brewed by the Kazbek variety (Table 5); this is apparently due to a significantly higher concentration of linalool whose sensory threshold in beer is 2,2 µg/L (Steinhaus a Schieberle, 2000). The bitterness decay curve and evaluation of bitterness character determined 20 to 60 seconds after swallowing a sip showed a slower decay of sensory bitterness and less gentle nature of bitterness character after drinking of beers hopped by Kazbek variety compared to Saaz (Figure 8). Based on the aroma and character of bitterness, a triangle test distinguished beers hopped by Kazbek from beers hopped by Saaz at the probability level P = 0.05 in two of three years, although the analytical bitterness of the beers was approximately the same.

Dry hopping: The results of sensory evaluation of experimental beers dry-hopped by Kazbek variety are shown in Table 6. Beers hoped at doses of 2.5 and 4.0 grams per liter and a 3-day contact time with hops were evaluated as best. Beers with higher doses of hops and longer

Table 3 *Results of analysis of kettle hopped beers*

		Saaz		Kazbek	
		R	SD	R	SD
Original extract	% w/w	12.2	0.3	12.2	0.1
Alcohol	% v/v	4.8	0.3	4.8	0.3
Apparent attenuation	%	74.8	3.4	74	4
pH		4.6	0.2	4.6	0.1
Color	EBC	10.1	0.5	9.7	0.3
Head retention (NIBEM)	s/30mm	306	19	308	10
Bitterness	IBU	33.1	4.2	32.9	2
Iso-alpha-acids	mg/L	33.3	0.2	32.7	0.1

R: mean value
SD: Standard deviation

Table 4 *The content of hop oils in kettle hopped beers (μg/L)*

	Saaz		Kazbek	
	R	SD	R	SD
α-pinene	1.36	1.6	1.63	2.1
β-pinene	0.20	0.2	0.21	0.1
Myrcene	3.11	1.9	5.83	4.7
Limonene	1.16	1.0	1.06	0.8
Linalool	17.99	8.7	47.36	24.4
β-caryophyllene	1.92	1.0	0.86	0.1
4-terpineol	1.84	0.2	1.37	0.5
β-farnesene	18.65	13.1	6.62	4.2
α-humulene	24.50	19.1	7.44	5.5
α-terpineol	24.40	28.9	7.73	2.1
cis-geraniol	2.24	1.1	3.21	1.9
α-ionon	0.62	0.4	0.39	0.2
β-ionon	0.53	0.1	0.36	0.1
α-iron	0.49	0.4	0.70	0.6
β-caryophyllenepoxid	1.77	0.3	4.83	5.0
Farnesol	37.63	19.6	54.46	15.9

R: mean value
SD: Standard deviation

contact times showed astringent and clinging bitterness, which most of the assessors evaluated negatively. This is due to the fact that dry-hopped beer is more prone to release water-soluble polyphenols and other hop substances that influence the intensity and character of the bitterness of beers (Parkin a Shellhammer, 2017). However, a detailed assessment shows that some evaluators (B, J, K) have preferred strongly hopped beers. This documents the individuality of sensory evaluation of beers in general.

4 Conclusion

The results of the brewery pilot tests have shown that Kazbek's aromatic hops offer very good sensory beer quality results both in kettle and dry hopping. Its use in brewing can be seen in dry hopping; thanks to the composition of hop oils it was included into the category of „flavor hops". Excellent sensory properties have already been demonstrated in numerous brewery tests in several breweries of varying sizes. In the next work we focus on the detailed study of hop oils and their profile in beer during dry hopping.

Table 5 *Results of sensory analysis of kettle hopped beers by a descriptive test*

	ŽPČ		Kazbek	
	R	SD	R	SD
Carbonation	2.9	0.08	2.93	0.09
Palate - fullness	2.93	0.13	2.93	0.05
Bitterness	3	0.16	2.96	0.05
Astringency	1.66	0.42	1.6	0.24
Sweetness	1.29	0.28	1.48	0.17
Sourness	0.93	0.33	1.03	0.26
Hoppy	0.9	0.08	1.55	0.35
Fruity / estery	1.29	0.1	1.26	0.12
Overall impression	3.7	0.49	3.8	0.65

R: mean value; SD: Standard deviation; Descriptors: ascending scale 0 - 5 (none - very strong); Overall impression. Descending scale 1–9 (1 - excellent; 9 - inappropriate)

Table 6 *Results of the sensory ranking test of beers dry hopped by Kazbek hops*

Contact time	3 days			12 days		
Hop dose(g/L)	1	2.5	4	1	2.5	4
A	4	5	6	1	2	3
B	2	4	1	2	3	6
C	4	5	6	3	2	1
D	5	6	4	1	2	3
E	3	5	4	6	2	1
F	4	6	5	3	2	1
G	4	6	5	3	2	1
H	3	5	6	2	4	1
I	4	5	6	3	2	1
J	1	3	2	5	6	4
K	1	4	3	2	5	6
Rank sums	35	54	48	31	32	28

A-K: Assessors

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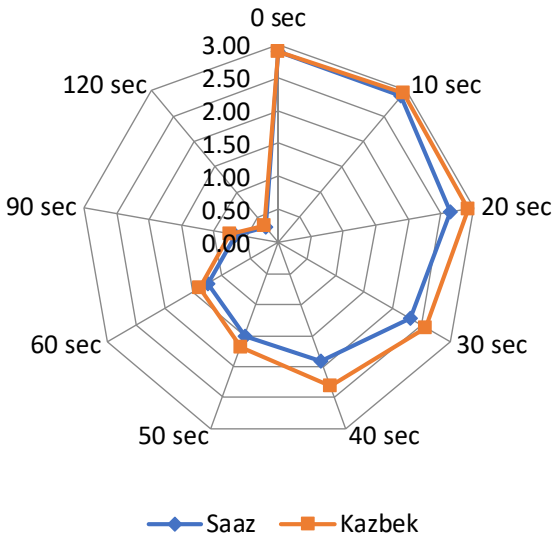

Figure 8 *Results of sensory analysis of beers – bitterness decay profile*

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