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Review

SAAZ—Fine Aroma Hop Pedigree: A Review of Current Knowledge

Jana Olšovská [1,*], Lenka Straková [2], Vladimír Nesvadba [3], Tomáš Vrzal [1], Michaela Malečková [1], Josef Patzak [3] and Pavel Donner [3]

[1] Research Institute of Brewing and Malting, Prague, Lípová 511/15, 120 00 Prague, Czech Republic; vrzal@beerresearch.cz (T.V.)
[2] Department of Crop Science, Breeding and Plant Medicine, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic; lenka.strakova@asahibeer.cz
[3] Hop Research Institute Co., Ltd., Saaz, Kadaňská 2525, 438 01 Žatec, Czech Republic; patzak@chizatec.cz (J.P.); donner@chizatec.cz (P.D.)
* Correspondence: olsovska@beerresearch.cz; Tel.:+420-724-597

Abstract: The hop variety Saaz is well known over the world and is usually used for brewing of lager beers. Recently, the new related varieties Saaz Late, Brilliant, Comfort, and Shine were registered. Information about these varieties is splintered and often available only in the Czech language in regional journals. This review (i) summarizes previously published data (breeding history, genetic data, basic parameters such as yield, sensory profile, concentrations of key technologically important hop compounds), (ii) presents long-term data (2004–2021), and (iii) shows similarities/differences among these varieties. All Saaz varieties are typically fine aroma hops with a relatively low content of alpha bitter acids ranging from 5 to 7 wt%, cohumulone amounts lower than 30% rel., and hop oil content of about 1.0 wt%. Even though the new varieties have no identical chemical parameters to the original Saaz, they can substitute this established standard as well. Furthermore, the varieties Saaz Comfort and Saaz Shine show high resistance to *Pseudoperonospora humuli* as well as very good tolerance to drought.

Keywords: hops; Saaz; breeding; bitter acids; hop oils; hop aroma; hop authenticity; draught tolerance; fungal resistance

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1. Introduction

Czech hops and especially the most famous representative Saaz variety (in Czech it is Žatecký poloraný červeňák or ŽPČ) are an integral part of traditional pilsner lager. The significance of this fine aroma variety is not only regional, but it is popular elsewhere, especially in Germany, Japan, and the U.S. Every year, about 8000 tons of this variety is grown in the Czech Republic on about 4200 hectares, of which about 75% are exported. Furthermore, the Saaz variety is also a basic material for breeding new related varieties. There are currently four other related varieties registered, namely Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz Shine.

The beginnings of breeding were based on a simple selection of populations of wild hops, which were originally used for beer brewing. In this way, the original regional varieties were obtained, such as Saaz, Auscha, "dubský", "hřebčí", etc. The quality of beer brewed from these regional varieties proved that the best hops come from the Saaz region. The founder of modern methods of hop breeding using clonal selection in the original regional stands was Karel Osvald. He was involved in clone selection from 1927 when 150 clones were selected, and then two seedlings were planted [1,2]. Due to Oswald's long-term efforts, the Czech hop industry obtained three clones, which were named after him. These three Oswald clones, clone 114, clone 31, and clone 72, were chosen by the leading growers themselves [3].

In the 1960s, hop hybridization, i.e., crossbreeding, began to be used in hop breeding. Bor and Sládek were the first registered Czech varieties bred by hybridization in 1994. In 1996, another variety, Premiant, was registered, and in 2001 the first Czech bitter hop variety, Agnus, which has an alpha acid content of 10%, was registered [4].

Hop breeding in the Czech Republic is mostly focused on aromatic hops, especially fine aroma ones. The basis of aromatic hop breeding is Saaz; however, in recent years, foreign varieties or even wild hops have also been used [5]. In 1995, a new program was launched to obtain aromatic hop varieties that will be of Saaz origin. Firstly, the fine aroma Saaz Late variety was registered (in 2011), and subsequently, the three new varieties Saaz Brilliant, Saaz Comfort, and Saaz Shine (in 2019), which show several identical features to Saaz, were registered [6]. Very detailed information about the cradle of Saaz hops was published in 2020. The author has mapped the history of hop cultivation in the Žatec region since the 19th century and the overall genesis of hop culture in Bohemia, Bavaria, and Western Europe [7].

1.1. Crossbreeding Methods of Saaz Varieties

Between 1997 and 2006, 72 crossbreedings were carried out. The seeds were obtained after suitable parent components were sown in a greenhouse; subsequently, the young plants (20,792 pieces) were tested for resistance to hop downy mildew and hop powdery mildew. The resistant and tolerant plants (6970 pieces) were planted in a breeding hop garden. Afterward, in the second year of cultivation, the best genotypes were selected for the second breeding stage plot. In total, 101 genotypes were selected, which represents only 0.49% of genotypes from the original number of genotypes obtained. Furthermore, after a five-year evaluation, the 16 best genotypes were propagated and planted in a nursery in three replicates and then in field and zoning experiments where a thorough evaluation in terms of growing and brewing characteristics was performed. These parameters were finally assessed during the registration trials of the nine best genotypes by the Central Institute for Supervising and Testing in Agriculture of the Czech Republic. New hop varieties were evaluated in breeding nurseries, field trials, and pilot plant areas. Due to the originality of each parent material, the performance of some hop varieties may be lower than those of virus-free stands [8]. Currently, each variety is monitored in maintenance breeding by a minimal number of 40 plants in such a way that 10 mothers are evaluated each year. In particular, each mother is evaluated in terms of morphological features, where deviations from the uniformity of a given hop variety are monitored.

During the registration trials as well as long-term monitoring, the quality of the variety was assessed using parameters such as hop yield, content, and composition of hop key compounds, such as resins [9] and essential oils [10]. Each plant was harvested separately, and their combing was performed on a stationary picking machine Wolf (Z + M Agro s.r.o., Czech Republic). The yield was given in kg of fresh hops per plant (hereinafter only in kg/plant). The conversion of hop yield was expressed by the number of plants per 1 ha that at a spacing of 1.14 × 3.00 m represents 2900 plants. The coefficient of dry matter in fresh hops and dry hops was four.

Monitored parameters (yield, concentrations of alpha and beta acids, cohumulone, colupulone, xanthohumol (X), and desmethylxanthohumol (DMX) were statistically processed, and average (A), median (Med), and standard deviation (s) were presented. The coefficient of variation (CV), showing the extent of variability in %, was used for a comparison of the variability of statistical features differing in a unit measure.

The evaluation of Saaz varieties was performed in locations with different soil and irrigation conditions given in Table 1.

Table 1. Locations of Saaz evaluation and their conditions.

Region Name	GPS	Type of Soil	Irrigation
Ohře river basin Rybňany Stekník	50°19′59.1″ N 13°35′47.9″ E 50°19′47.7″ N 13°37′05.4″ E	Brown	yes
Rakovník region Nesuchyně Chrášťany	50°10′55.4″ N 13°41′38.8″ E 50°09′53.8″ N 13°39′18.9″ E	Brown	no
Golden Creek Valley—Očihov	50°11′30.0″ N 13°28′39.9″ E	Permian red	no
Auscha region Radovesice	50°24′08.0″ N14°04′06.4″ E	Permian red	no

1.2. Fine Aroma Hop Varieties

All of the world's hop varieties listed in the "Hop Variety List" are currently divided into aromatic, bitter, and others [11], which is based on their usage in the brewing industry [12,13]. However, some information about detailed necessary distribution is available. This categorization was set by the EEC in 1971 [14]. Forster et al. dealt with this problem at an IHGC conference in Spain, where he discussed whether distinguishing hop varieties as either aroma or bitter hops is still relevant: "No hops in existence contain only aroma substances or only bitter substances" [15]. For example, in Germany in 1970, the concentration of α acids in bitter varieties ranged from 7.0 to 9.2 while in aroma varieties from 3.1 to 4.1 [16]. In general, aroma varieties contain low concentrations of bitter acids but enhance beer quality due to their quality. Since aroma hops had an elegant and fine smell and bitter varieties were described as strong and aggressive, distinguishing them was rather clear [15].

After 2000, the situation changed radically. Many new varieties cannot be unanimously classified within these two categories. Minimally, aroma categories such as "flavor" and "fine aroma" hops should be added. "Flavor" varieties have a fruity character and are often used for dry hopping. "Fine aroma" varieties are original landraces from Central Europe and should be a subgroup of the aroma group. Forster proposed to include old landraces, such as Hallertauer, Hersbrucker, Saaz, Spät, and Tettnanger, in this subgroup, as well as closely related varieties such as Saphir and Spalter Select. He defined the key parameters of this subgroup as α acids, β/α, cohumulone ratio, and hop oil concentration <7, 0.8–2.5, <28, and <1.2, respectively [15].

A designation of "fine aroma hops" or "noble hops" can also be found in previous studies. Vent and Vent, in 1999 suggested a new classification of hop varieties, which sorted them into four groups—fine aroma, aroma, bitter (dual-purpose), and high-alpha ones [17].

Krofta also described the "fine aroma" subgroup in his study [10]. He compared several aroma varieties including three original landraces from Central Europe named according to the region of their cultivation, Saaz, Tettnang, and Spalter. He processed results from 1995 to 2002 and concluded that fine aroma varieties had a similar composition of hop resins. The content of α bitter acids was mostly in the range of 3–4% w/w, and the ratio of α/β bitter acids was lower than 1.0, which is typical of these hops. The cohumulone ratio was in the range of 23–26% rel., and the colupulone ratio was in the range of 39–43% rel. [10].

Explaining the chemical nature of a fine hoppy aroma is a very complex and a long-term process in which many scientists have been involved for more than 50 years. Although there has been progress, the statement of Meilgaard at the EBC Symposium on Hops in 1994, "A fine hop aroma, especially the so-called noble hop aroma, is still an elusive quality. Despite 40 years of research, we still do not know what compounds cause it", is still valid. [18]. The "noble" or "fine hoppy aroma" character is usually described as herbal, floral, and spicy. Monoterpene alcohols including linalool, geraniol, and citronellol

and several esters of linalool and geraniol are responsible for the floral and/or citrusy aroma [19–25]. The essence of herbal and spicy aroma was studied on the varieties Saaz, Spalter Select, Tettnanger, Hersbrucker, and Perle. The authors concluded that the herbal and spicy aroma significantly depends on natural sesquiterpenoids, mainly caryophyllene epoxide, humulene epoxide I, humulene epoxide II, and humulenol II that are transformed into their oxygenated forms during brewing [26]. According to our new study, farnesol, which is an oxidized form of farnesene, is responsible for the characteristic fine hoppy aroma in lager beers hoped by Saaz family varieties [27].

Alberts named the Saaz hops varieties "the Queen of Hops" based on history and genetics. This variety is the mother of fine aroma hops, such as Spalt, Tettnang, and Lubelski, as well as some cultivars from Ukraine and Russia [28].

This review aims to summarize information published on the new related varieties Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz. No study has been yet published comparing all these new varieties with the traditional Saaz variety. This review (i) summarizes previously published data (breeding history, genetic data, basic parameters such as yield, sensory profile, and concentrations of key technologically important hop compounds), (ii) presents long-term data (2004–2021) regarding the content of α acids, β acids, xanthohumol, desmethylxanthohumol, and relative content of cohumulone and colupulone as well as essential hop oils, and (iii) shows similarities/differences among these varieties. The next goal of the work is to highlight the necessity of officially recognizing a new subgroup of aroma hops such as "fine aroma hops". Last but not least, this non-traditional review is supplemented by author's data, which include, in addition to the long-term monitoring of contents of basic chemical substances, the family's resistance to Pseudoperonospora humuli, and drought, which is a manifestation of global climate change.

The determination of hop resins and hop oils in hops was performed according to the commonly used EBC method [29] and previously published study [10]. These data are the property of the Hop Institute in Žatec, CZ, and the Research Institute of Brewing and Malting in Prague, CZ, where the authors of the article work. The evaluation of a hop aroma takes place in Žatec every year after the harvest by at least 100 evaluators. The data are the property of the Hop Institute in Žatec, CZ.

2. Pedigree of New Saaz Varieties

The pedigree and the registration year of Saaz varieties are given in Table 2. Their hop cones are documented in Figure 1. More detailed characteristics of individual varieties are given in the following text.

Table 2. Pedigree of new Saaz varieties.

Variety	Pedigree	Registration Year and Owner
Saaz Late	Breeding material originating from Saaz semi-early red bine hop × open pollination	2011, Hop Research Institute, Co., Ltd., Žatec, Czech Republic
Saaz Brilliant	Selection from hybrid progeny after mother Saaz × male Saaz (inzucht crossing)	2019, Hop Research Institute, Co., Ltd., Žatec, Czech Republic
Saaz Comfort	Hybrid progeny after mother Serebrianka (Russia) × male Saaz	2019, Hop Research Institute, Co., Ltd., Žatec, Czech Republic
Saaz Shine	Selection from hybrid progeny after mother Sladek × male Saaz.	2019, Hop Research Institute, Co., Ltd., Žatec, Czech Republic

Figure 1. Hop cones of Saaz varieties.

2.1. Saaz Late

2.1.1. Breeding Method

Saaz Late was bred by the selection of the F1 generation progeny that had its origin in Saazer (see Table 2). The progenies were artificially infected with powdery mildew (*Podosphaera humuli*) under glasshouse conditions. Resistant plants were planted onto our breeding plot and tested for resistance to downy mildew (*Peronoplasmopara humuli*). The selected clone 4237 was planted onto the second breeding stage plot. As the clones displayed good parameters after four years' assessment within this stage, they were propagated and planted onto the third breeding stage plots in three replicates to examine the stability of demanded breeding characteristics. Brewing tests in a pilot brewery were carried out and the clones were further propagated for field trials on Stekník hop farm in 2007. In 2008, they were included in registration trials, and in 2010 it was registered as the variety Saaz late Ametyst, which was renamed to Saaz Late in 2011 [30].

2.1.2. Characteristic of Variety

During the registration trial, the content of alpha and beta acids was 3.5–6.0% and 4.0–6.5%, respectively. A ratio of alpha/beta acids ranging from 0.8 to 1.0 was the main reason for why the cultivar was included in the aroma hop group. The cohumulone ratio ranged between 20% and 25% rel., whereas the colupulone ratio was between 39% and 43% rel. [30].

The long-term data from 2004 to 2021 are given in Table 3. The average and median content of alpha acids were 3.12% and 2.92 wt%, respectively, with variability of 1.00%. The average and median content of beta acids were 4.52% and 4.60 wt%, respectively, with variability of 0.67%, resulting in an average ratio of alpha/beta content of 0.68 with variability of 25.02%. The corresponding average amount of cohumulone and colupulone was 23.96% and 41.51%, respectively. The average and median content of xanthohumol, as well as DMX concentration, were nearly identical, namely 0.27 wt% and 0.06 wt% with variability of 18.66% and 27.76%, respectively. Furthermore, the content of polyphenols ranged from 2.5 to 3.0 wt%.

The average yield, 2.0–2.6 t/ha of dry cones, was significantly higher in comparison with the standard variety Saaz (0.8–1.2 t/ha). Saaz Late has vigorous growth and an irregular cylindrical shape. Cones begin to grow 2.5–3.0 m above ground level. Hop cones are firm and easy to pick, which help to decrease losses during harvest. They are of medium size and have an oblong shape, the rachis is regular and 15–17 mm long (see Figure 1). The violet bine is 10–13 mm thick, and fertile laterals are very long. The weight of 100 cones is about 10–15 g. The vegetation period lasts for 128–135 days [30].

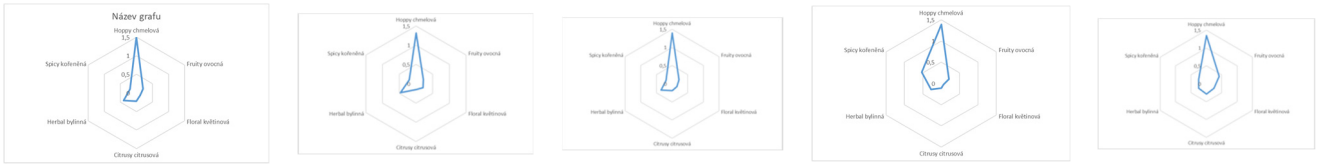
Table 3. Saaz Late—The content of hop resins, xanthohumol (X), and desmethylxanthohumol (DMX) in the years 2004 to 2021.

Parameter	α Acids (wt%)	β Acids (wt%)	α/β	Cohumulone (% rel.)	Colupulone (% rel.)	X (wt%)	DMX (wt%)
A	3.12	4.52	0.68	23.96	41.51	0.27	0.06
Med	2.92	4.60	0.67	23.50	40.80	0.26	0.06
S	1.00	0.68	0.17	1.98	4.756	0.05	0.02
CV (%)	32.05	15.05	25.02	8.25	11.46	18.66	27.76
Min	1.50	3.48	0.38	22.20	34.80	0.18	0.04
Max	4.67	5.90	0.99	29.50	53.40	0.38	0.09

A, average; Med, median; S, standard deviation; CV, coefficient of variation; Min, minimal content; Max, maximal content; X, xanthohumol; DMX, desmethylxanthohumol.

2.1.3. Aroma

The Saaz Late variety has a characteristic fine hoppy aroma (1.32) with a pronounced herbal (0.46) and floral (0.32) scent and a weaker fruity (0.22), spicy (0.20), and citrusy (0.15) scents in the background (see Figure 2, intensity of aroma is given on a scale of 0–1.5).



Saaz (original) Saaz Late Saaz Brilliant Saaz Comfort Saaz Shine

Figure 2. Aroma of Saaz varieties.

2.2. Saaz Brilliant

2.2.1. Breeding Method

Saaz Brilliant was bred by the selection of hybrid progeny after mother Saaz × male Saaz (inzucht) crossing. The variety was registered in 2019 in the Czech Republic.

2.2.2. Characteristic of Variety

During the registration trial in 2013–2018, the average content of alpha and beta acids was 4.1 and 4.0 wt%, respectively. The cohumulone ratio was about 26% rel. [31].

The long-term data from 2005 to 2021 are given in Table 4. The average content of alpha acids is 3.80 wt% with variability of 19.60%, which is influenced by year and location as well as the alpha/beta acid ratio. While the average ratio of this variety is 1.42, in 2015 and 2014 the average alpha/beta ratio was 1.67 and 0.98, respectively. The cohumulone content, represented by the average and median, is 24.53% rel. and 24.32% rel., with variability of 8.99%. The lowest cohumulone content was determined in 2015 (20.80% rel.), whereas a maximal cohumulone content above 30% was determined in 2011. The average and median content of colupulone are 44.55% rel. and 44.62% rel., respectively, with variability of only 6.11%. The lowest and highest colupulone contents were determined in the same samples as the corresponding cohumulone ones. The average and median content of xanthohumol as well as DMX were identical, 0.21 and 0.04 wt%, with variability of 19.67% and 49.81%, respectively. The very high variability of DMX was very probably caused by its low concentration [32].

The average yield was 1.8–2.3 t/ha of dry cones. Saaz Brilliant has a regular cylindrical shape. Cones begin to grow 1.0–1.5 m above the ground level. Hop cones are firm and easy to pick, which help to decrease losses during harvest. They are of medium size, have an oblong shape, and the rachis is regular and 18–20 mm long. The green-red bine is 12–15 mm thick, and fertile laterals are medium length. The weight of 100 cones is about 12–17 g.

The vegetation period lasts for 123–130 days [32] (new data). Finally, the concentrations of polyphenols in 2018–2021 were 4.0–5.0%.

Table 4. Saaz Brilliant—The content of hop resins, xanthohumol (X), and desmethylxanthohumol (DMX) in the years 2004 to 2021.

Parameter	α Acids (wt%)	β Acids (wt%)	α/β	Cohumulone (% rel.)	Colupulone (% rel.)	X (wt%)	DMX (wt%)
A	3.80	2.73	1.42	24.53	44.55	0.21	0.04
Med	3.71	2.77	1.40	24.32	44.62	0.21	0.04
S	0.74	0.53	0.26	2.20	2.72	0.04	0.02
CV (%)	19.60	19.28	18.13	8.99	6.11	19.67	49.81
Min	2.38	1.60	0.97	20.80	39.50	0.13	0.02
Max	5.36	4.03	2.22	31.30	50.80	0.32	0.14

A, average; Med, median; S, standard deviation; CV, coefficient of variation; Min, minimal content; Max, maximal content; X, xanthohumol; DMX, desmethylxanthohumol.

2.2.3. Aroma

Saaz Brilliant has a characteristic fine hoppy aroma (1.4) with a pronounced herbal scent (0.35) and weak fruity (0.22), citrusy (0.19), and spicy (0.19) scents in the background (see Figure 2, intensity of aroma is given on a scale of 0–1.5).

2.3. Saaz Comfort

2.3.1. Breeding Method

Saaz Comfort originates from the selection of hybrid progeny after mother Serebrianka (Russia) × male Saaz crossing.

2.3.2. Characteristic of Variety

During the registration trial in 2013–2018, the average content of alpha and beta acids was 6.4 and 6.5 wt%, respectively. The cohumulone ratio was about 18% rel. [31].

The long-term data from 2005 to 2021 are given in Table 5. The Saaz Comfort variety has an average and median content of alpha acids of 5.60 and 5.59 wt%, respectively, with variability of 18.16%. During this period, the lowest concentrations of alpha acids were determined in 2018 (3.39 wt%) and the highest in 2005 (7.80 wt%).

Table 5. Saaz Comfort—The content of hop resins, xanthohumol (X), and desmethylxanthohumol (DMX) in the years 2004 to 2021.

Parameter	α Acids (wt%)	β Acids (wt%)	α/β	Cohumulone (% rel.)	Colupulone (% rel.)	X (wt%)	DMX (wt%)
A	5.60	5.54	1.06	18.00	37.20	0.34	0.14
Med	5.59	5.44	1.07	17.90	36.65	0.33	0.14
S	1.02	1.28	0.26	2.00	2.80	0.07	0.06
CV (%)	18.16	23.20	24.48	11.21	7.53	19.59	42.69
Min	3.39	3.14	0.50	14.80	32.70	0.21	0.05
Max	7.90	8.57	1.58	26.20	51.20	0.64	0.34

A, average; Med, median; S, standard deviation; CV, coefficient of variation; Min, minimal content; Max, maximal content; X, xanthohumol; DMX, desmethylxanthohumol.

As follows from Table 5, the long-term concentration of beta acids is very similar to alpha acids (average 5.54 wt%, median 5.44 wt%, variability 23.20%). Therefore, the average and median of the alpha/beta ratio are nearly identical (1.06 and 1.07, respectively).

However, the variability of this value is 24.48%. The explanation follows from the long-term individual annual data). For example, in two consecutive years, namely in 2014 and 2015, the average alpha/beta ratio was 0.73 and 1.24. The average content of cohumulone is 18.00% rel. and the median is 17.90% rel., with a variability of 11.21%. The highest cohumulone content was determined in 2015 (26.20% rel.) and the lowest in 2018 (14.80% rel.). The variability of the cohumulone content is 11.02%. However, compared to the Saaz Brilliant variety, there are no substantial differences in average cohumulone contents among the individual years. The average colupulone content is 37.20% rel. and the median is 36.65% rel., with a variability of 7.53%. The content of xanthohumol, expressed as average and median, were almost identical at 0.34 wt% and 0.33 wt%, respectively, with a variability of 19.59%. This amount is higher in comparison with Saaz Brilliant, as well as the concentration of DMX, where the average and median were identically 0.14 wt% with variability of 42.69% (Nesvadba and Charvátová 2020 and new authors data).

The average yield was 2.1–2.5 t/ha of dry cones. Saaz Comfort has a regular cylindrical shape. Cones begin to grow 0.5–1.5 m above the ground level. Hop cones are firm and easy to pick, which helps to decrease losses during harvest. They are medium to high and have an oblong shape, and the rachis is regular and 20–23 mm long. The green-red bine is 11–14 mm thick and fertile laterals are medium length. The weight of 100 cones is about 14–21 g. The vegetation period lasts for 124–132 days [31] (new authors data). Finally, the concentrations of polyphenols in 2018–2021 were 4.0–5.0%.

2.3.3. Aroma

Saaz Comfort has a characteristic fine hoppy aroma (1.39) with a pronounced spicy (0.53) and herbal (0.28) scent and a weak fruity (0.21) scent in the background with traces of citrusy (0.11) and floral (0.06) aromas (see Figure 2, intensity of aroma is given on a scale of 0–1.5).

2.4. Saaz Shine

2.4.1. Breeding Method

Saaz Shine originates from the selection of hybrid progeny after mother Sladek × male Saaz crossing.

2.4.2. Characteristic of Variety

During the registration trial in 2013–2018, the average content of alpha and beta acids was 3.7% and 3.8%, respectively. The cohumulone ratio was about 24% rel. [30].

The long-term data from 2005 to 2021 are given in Table 6. The Saaz Shine variety has an average and median content of alpha acids of 3.62 wt% and 3.43 wt%, respectively, with variability of 28.91%. The lowest and highest concentrations of alpha acids were recorded in 2018 and 2021, namely 1.71 and 5.89 wt%, respectively. The average and median content of beta acids were 2.87 and 2.95 wt%, respectively, with variability of 25.19%. The lowest concentrations of beta acids were in 2007 and 2018 at 1.15 and 1.98 wt%, respectively. The ratio of alpha/beta acids expressed as the average and median in a long-term perspective was 1.28 and 1.22, respectively, with a variability of 22.98%. The highest ratio was recorded in 2007 (2.39) and the lowest in 2009 (0.88). The long-term average content of cohumulone, median, and variability are 24.40 wt%, 24.30 wt%, and 24.30%, respectively. The highest amount was determined in 2019 and 2021, where both were 27.60 wt%. The lowest amount was recorded in 2019 as 20.30 wt%. The long-term average content of colupulone, median, and variability are 45.37 wt%, 45.00 wt%, and 7.43%, respectively. As well as Saaz Late, Saaz Brilliant, and Saaz Comfort, the Saaz Shine variety has a low content of xanthohumol and DMX (0.36 and 0.04 wt%, respectively).

Table 6. Saaz Shine—The content of hop resins, xanthohumol (X), and desmethylxanthohumol (DMX) in the years 2004 to 2021.

Parameter	α Acids (wt%)	β Acids (wt%)	α/β	Cohumulone (% rel.)	Colupulone (% rel.)	X (wt%)	DMX (wt%)
A	3.62	2.87	1.28	24.40	45.37	0.36	0.04
Med	3.43	2.95	1.22	24.30	45.00	0.38	0.04
S	1.04	0.72	0.29	1.77	3.34	0.08	0.01
CV (%)	28.91	25.19	22.89	7.25	7.43	20.57	31.25
Min	1.71	1.15	0.88	20.30	38.80	0.21	0.02
Max	5.89	4.26	2.39	27.60	51.60	0.56	0.07

A, average; Med, median; S, standard deviation; CV, coefficient of variation; Min, minimal content; Max, maximal content; X, xanthohumol; DMX, desmethylxanthohumol.

The average yield was 2.0–2.4 t/ha of dry cones. Saaz Shine has a regular cylindrical shape. Cones begin to grow 0.5–1.0 m above the ground level. Hop cones are firm and easy to pick, which helps to decrease losses during harvest. They are of medium size and have an oblong shape, and the rachis is regular and 16–20 mm long. The green-red bine is 9–12 mm thick and fertile laterals are medium length. The weight of 100 cones is about 11–17 g. The vegetation period lasts for 124–132 days. Finally, the concentrations of polyphenols in 2018–2021 were 3.5–4.5%.

2.4.3. Aroma

Saaz Shine has a rich but still fine hoppy aroma (1.35) with a pronounced fruity (0.42) and citrusy (0.29) scent followed by a balanced herbal, floral, and spicy scent (0.25) in the background (see Figure 2, intensity of aroma is given on a scale of 0–1.5).

3. Genetic Analysis of Saaz Varieties

The authenticity and control of cultivar purity of hops is a significant topic in the hop industry. Although every hop variety has a characteristic chemical profile of bitter acids, hop oils, and polyphenols resulting in typical varietal bitterness and aroma, the composition of chemical compounds is influenced by growing season, locality, technology, and environmental conditions. Therefore, it may be sometimes difficult to recognize or confirm the variety based solely on these chemical parameters. Fortunately, molecular genetic methods based on DNA provide a reliable tool for the evaluation of individual cultivars and genotypes. A very efficient marking system for genotyping and authenticity control of Czech hop cultivars based on EST-SSR was also applied for the description of Saaz varieties. It is a successful and efficient system that is used not only for cultivar determination but also for the evaluation of molecular genetic variability with addition of highly polymorphic molecular markers [32].

DNAs for genetic diversity analyses were isolated from young leaves, dried cones, or pellets of 250 world cultivars by the CTAB method [33]. Molecular analyses of 16 microsatellite loci were carried out according to Patzak et al. [33]. A dendrogram was based on Jaccard's similarity coefficient of 155 EST-SSR polymorphic molecular markers, determined by the Neighbor-Joining (NJ) method of the Unweighted Pair Group Method with Arithmetic means (UPGMA) in DARwin v. 5.0.155 (Dissimilarity Analysis and Representation for Windows, ADDED: accessed on 30 April 2018) software, visualized by Geneious Pro 4.8.2 (Biomatters Ltd., Auckland, New Zealand) software [34].

The resulting dendrogram, which corresponds to the genealogical, geobotanical, and chemical profile of bitter acids, hop oils, and polyphenols, is given in Figure 3. The Saaz and other genetically related varieties form a separate group among the aromatic hops. The category of aroma varieties comprises two subgroups of hops, to the first of which

belong fine aroma hops including Saaz and original landraces from Central Europe named according to the region of their cultivation.

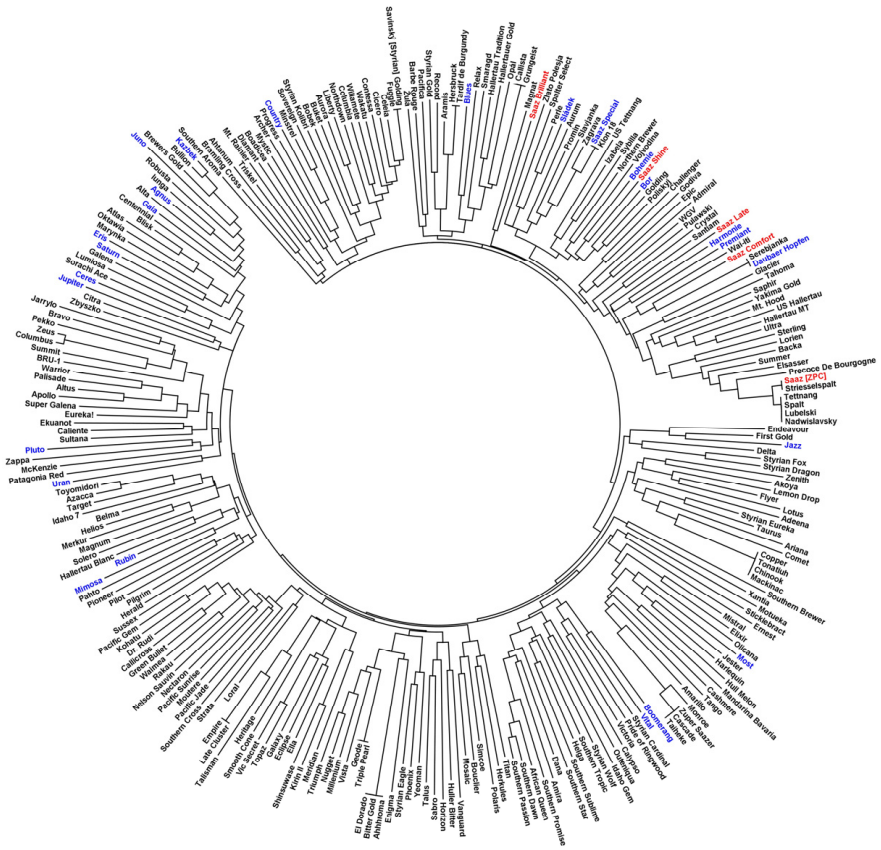


Figure 3. Dendrogram of genetic distances for 250 world hop cultivars (Czech hop cultivars are labeled in blue, and studied Saaz hop cultivars are labeled in red).

4. Resistance to Fungal Diseases

Within the breeding process, parameters such as agrotechnical aspects, storage ability, productivity, the content of alpha acids or interesting profile of hop oils, as well as resistance to fungal diseases and pests are demanded [35]. Resistant varieties require a substantially smaller extent of chemical protection, which considerably increases the quality of the product as well as economic benefits. Downy mildew (caused by the fungus-like (oomycete) organism *Pseudoperonospora humuli*) is a common fungal disease affecting hops and is common in wetter climates. This pathogen is typical of spike-like shoots. During spring (April and May), affected shoots grow from an infected hop rhizome and cause a primary infection. Consequently, plant sprouts are damaged resulting in gradual drying. Furthermore, the secondary infection attacks buds, apical meristems, hop inflorescences, cones, and leaves, which finally go brown. As the affected cones are damaged, the aroma and content of bitter acids decrease [36]. The resistance of Saaz varieties was evaluated according to the published method, which was also used for the other Czech varieties [37]. The results are summarized in Table 7. The original Saaz, as well as the Saaz Brilliant variety, has medium resistance. Saaz Late has low and medium resistance to primary and secondary infection, respectively. Saaz Comfort obtained from its mother (Serebrjanka) has high resistance to both infection types.

Table 7. The resistance of the Saaz varieties to *Pseudoperonospora humuli*.

	Primary Infection	Secondary Infection
Saaz	Medium	Medium
Saaz Late	Low	Medium
Saaz Brilliant	Medium	Medium
Saaz Comfort	High	High
Saaz Shine	High	Medium

5. Tolerance to Drought

The period from 2011 to 2020 was the warmest decade in recorded history. The average global temperature in 2019 was 1.1 °C above pre-industrial levels. Human-induced global warming is currently increasing at a rate of 0.2 °C per decade [38]. This change is associated with an alarming lack of rainfall and surface and groundwater supplies. This phenomenon can negatively influence hop yield and its quality [39]. Moreover, hop cultivation areas without any irrigation are endangered and threatened with extinction. This problem could be solved by planting hops with varieties or genotypes tolerant to drought [40]. Therefore, Czech hop breeding targets drought-tolerant genotypes with high crop yield and stability of quantitative and qualitative parameters, which will also be well-usable in the Czech brewing industry. Thus, Saaz varieties were tested for drought tolerance using a previously published method [40]. Five plants of each genotype were propagated and planted in 5 L pots in a standard growing substrate. Three plants in the most similar growth phase were selected for the measurement. The initial measurement of all genotypes occurred on irrigated plants that were not drought-stressed. After the initial measurement, their irrigation was terminated and the effect of drought stress on the visual and physiological manifestations of the plants was subsequently monitored. Ten days after the first measurement, the same plants were measured again. The rates of photosynthesis and transpiration were determined after being exposed to water and also partially to heat stress (greenhouse conditions), when the plants could not compensate the heat stress by cooling down due to irrigation. The results are summarized in Table 8 (new authors data).

Table 8. Draught tolerance.

	Initial Measurement			Measurement after 10 Days of Stress		
	A (µmol $CO_2/m^2/s$)	B (mmol $H_2O/m^2/s$)	V	A (µmol $CO_2/m^2/s$	B (mmol $H_2O/m^2/s$)	V
Saaz	8.23	2.13	2	0	0.25	4
Saaz Late	10.12	3.46	1	0	0.42	3
Saaz Brilliant	7.31	1.98	1	0	0.33	4
Saaz Comfort	7.65	2.34	1	1.48	0.51	2
Saaz Shine	9.78	2.02	1	3.11	0.78	2

A—rate of photosynthesis (µmol $CO_2/m^2/s$), B—transpiration rate (mmol $H_2O/m^2/s$), V—visual of plant: (1)—negligible growth retardation, slightly yellowish lower leaves, drought symptoms were minimal; (2)—slowed growth, plants showed yellowish lower leaves, fast regeneration after watering; (3)—wilted leaves, lower leaves dried up, stopped growth, plant regenerated and continued to grow after watering; (4)—completely dried up plants, no regeneration after watering.

The table shows that even after 10 days of water stress, the varieties Saaz Comfort and Saaz Shine show a photosynthesis rate of 1.48 µmol $CO^2/m^2/s$ and 3.11 µmol $CO_2/m^2/s$, respectively. The rate of the other genotypes is 0.00 µmol $CO_2/m^2/s$ as these plants dried out.

6. Comparison of New Saaz Varieties with Original Saaz

The original Saaz variety is a traditional fine aroma hop with a typical fine hoppy aroma (1.50) with a pronounced herbal (0.40) scent and weak citrusy (0.22), fruity (0.21), spicy (0.20), and floral (0.12) scent in the background. (see Figure 2). It has the lowest yield compared to the other Saaz varieties; however, it is still the most cultivated variety due to its excellent character [41]. Therefore, it is worth noting that the Saaz hop variety is protected by the EU legislation and obtained a Protected Designation of Origin (PDO) [42]. The long-term data of key compounds are given in Table 9 together with the parameters of all Saaz varieties for better clarity. The average alpha acid and beta acid content is 3.24 and 4.17%, respectively, which results in a balanced rate of alpha and beta acids (0.85), which causes, together with the unique content of hop oils, the inimitable character of the beer taste. The concentration of cohumulone and colupulone is 22.95 and 39.92%, which is very similar to Saaz Late, as well as the concentration of xanthohumol and DMX also. Moreover, the original Saaz variety has the highest total concentration of polyphenols, ranging from 5.5 to 6.5 in 2018–2021.

Table 9. Comparison of average parameters of Saaz varieties.

Parameter	Yield (t/ha)	α Acids (wt%)	β Acids (wt%)	α/β	Cohumulone (% rel.)	Colupulone (% rel.)	X (wt%)	DMX (wt%)
Saaz	1.65	3.24	4.17	0.85	22.95	39.92	0.30	0.07
Saaz Late	2.06	3.12	4.52	0.68	23.96	41.51	0.27	0.06
Saaz Brilliant	1.93	3.80	2.73	1.42	24.53	44.55	0.21	0.04
Saaz Comfort	2.16	5.60	5.54	1.06	18.00	37.20	0.34	0.14
Saaz Shine	2.21	3.62	2.87	1.28	24.40	45.37	0.36	0.04

X, xanthohumol; DMX, desmethylxanthohumol.

Saaz Comfort has the highest concentration of both alpha and beta acids; however, interestingly, has the lowest concentration of cohumulone. Finally, the ratio of alpha/beta acids of Saaz Brilliant (1.42) together with the highest concentration of colupulone is distinct in comparison with the original Saaz variety.

Finally, the knowledge of hop oils content in hop varieties is a crucial term for the aroma hop, related to utilization in brewing. Therefore, long-term monitoring (2005–2021) of hop oils was performed and results are summarized in Figure 4 and Table 9. The varieties Saaz Comfort and Saaz Shine have the highest average essential oil contents, namely 0.84 and 0.75 wt%, respectively, and no significant difference in essential oil content was determined between these varieties (*t*-test, $\alpha = 0.01$). Furthermore, a significant difference was found between the hop oil content of Saaz Late and Saaz Comfort, where the content is significantly lower in Saaz Late (0.66 wt%); however, the difference between Saaz Late and Saaz Shine was not proved. Saaz Brilliant (0.52 wt%) and original variety Saaz (0.51 wt%) have the lowest concentrations of hop oils among all Saaz, between which no statistically significant difference was found.

Table 10 displays the monitoring of hop oils in more detail, especially the relative concentration range of myrcene, caryophyllene, β-farnesene, humulene, and the sum of selinenes together with the range of the total sum of hop oils (new data). The varieties Saaz Comfort as well as Saaz Shine show the total content of essential oil even above 1% wt, which correlates with their intensive hop aroma. It is evident from the intervals of myrcene values that all varieties can have a myrcene content above 30% rel., and even the Saaz Late variety exceeded this value to 40% rel. in 2016 and 2017. Furthermore, the amount of caryophyllene and β-farnesene are almost on the same level among all evaluated varieties. The concentration of humulene is provably maximal in the varieties of Saaz and Saaz Shine. The concentration of selinene is statistically significantly the highest in Saaz Comfort ($\alpha = 0.01$). These results are in agreement with the independent study, where the

average content and composition of hop oils within monitoring performed from 2011 to 2020 were presented [43].

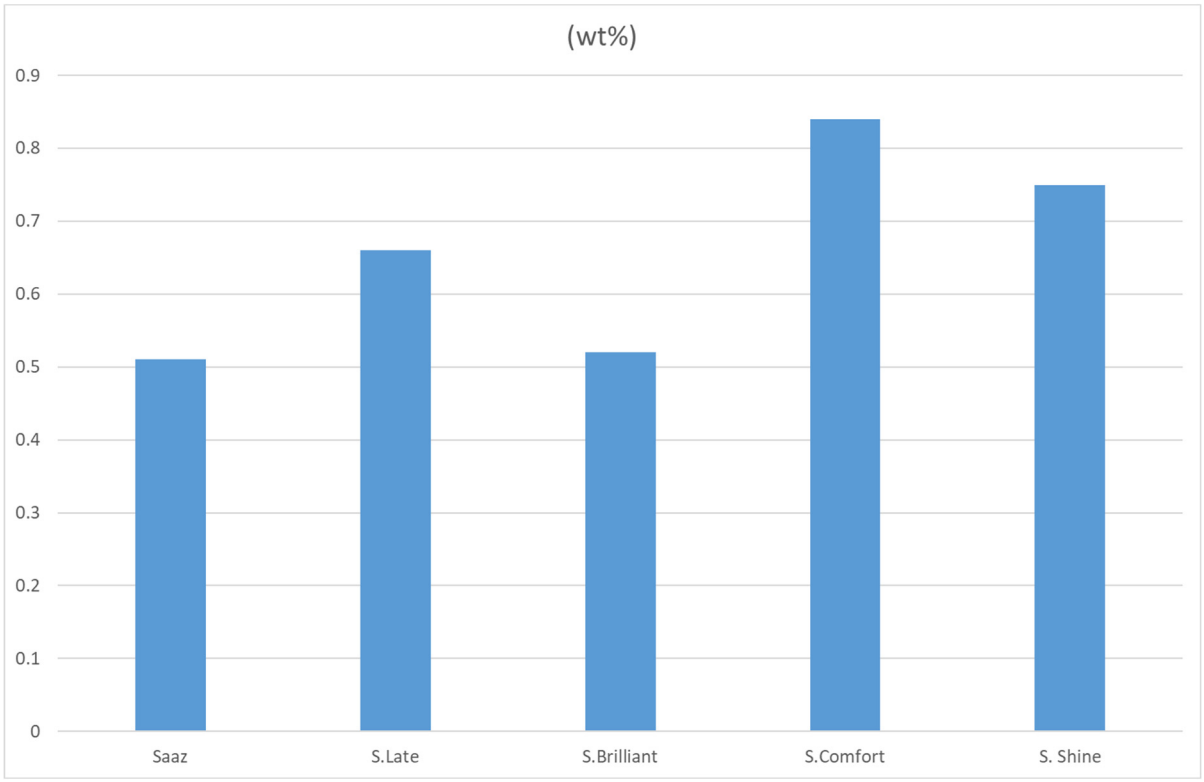


Figure 4. Average content of essential oils in new aromatic varieties and the original variety.

Table 10. Content and composition of hop essential oils in the years 2005 to 2021.

Parameter	Saaz	Saaz Late	Saaz Brilliant	Saaz Comfort	Saaz Shine
Sum of hop oils (wt %)	0.3–0.9	0.4–0.9	0.3–0.8	0.4–1.1	0.5–1.3
Myrcene (% rel.)	11–32	25–43	11–34	14–36	12–35
Caryophyllene (% rel.)	5–12	4–11	7–14	6–12	7–15
Farnesene (% rel.)	8–20	7–15	7–17	2–14	7–20
Humulene (% rel.)	15–43	10–20	14–33	10–20	22–39
Selinenes (% rel.)	1–6	3–8	1–9	11–26	<3

7. Conclusions

Saaz hops are one of a few traditional European hops, as well as Spalt, Hersbruck, Hallertau (Bavaria, Germany), Tettnang (Württemberg, Germany), and Lubelski (Lublin Poland). These varieties are called "landraces" since their names refer to the regions and places of origin. They have historical roots, dating back to pre-modern times. They are also called "fine aroma hops" or "noble hops" because of their pedigree and aroma profile, which include, in general, mainly herbal, spicy, and floral aromas in various proportions. Varieties then differ in the presence of other aromas of lower intensity, such as fruity and citrusy. Sometimes, woody or earthy scents are mentioned [44–47]. However, it is difficult to compare absolute aromatic profiles as there is no uniform nomenclature neither in science nor in the market, and it is impossible to find the same rating for a given variety. Of course, climate and weather play a key role in the resulting aroma.

Breeding of new hop varieties is accelerated not only by consumers' interest in a new hop aroma of beer but mainly due to decreasing tolerance of traditional varieties to pests and climate change. This necessity reflects a breeding program in the Czech Republic, which has a long-term tradition. It is focused on aromatic hops, especially "fine aroma ones". During 2011–2019, new varieties, such as Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz Shine, were registered.

The original Saaz is a traditional fine aroma hop with a typical fine hoppy aroma with a pronounced herbal scent and weak citrusy, fruity, spicy, and floral scents in the background. The long-term average contents of α acids, β acids, cohumulone, colupulone, and hop oils are 3.24 wt%, 4.17 wt%, 22.95% rel., 39.92% rel., and 0.51 wt%, respectively.

Saaz Late has a characteristic fine hoppy aroma with a pronounced herbal and floral scent and weak fruity, spicy, and citrusy scents in the background. The long-term average contents of α acids, β acids, cohumulone, colupulone, and hop oils are 3.12 wt%, 4.52 wt%, 23.96% rel., 41.51% rel., and 0.66 wt%, respectively.

Saaz Brilliant has a characteristic fine hoppy aroma with a pronounced herbal scent and weak fruity, citrusy, and spicy scents in the background. The long-term average contents of α acids, β acids, cohumulone, colupulone, and hop-oils are 3.80 wt%, 2.73 wt%, 24.53% rel., 44.55% rel., and 0.52 wt%, respectively.

Saaz Comfort has a characteristic fine hoppy aroma with a pronounced spicy and herbal scent and weak fruity scent in the background with traces of citrusy and floral scents. The long-term average contents of α acids, β acids, cohumulone, colupulone, and hop-oils is 5.60 wt%, 5.54 wt%, 18.00% rel., 37.20% rel., and 0.84 wt%, respectively.

Saaz Shine has a rich but still fine hoppy aroma with a pronounced fruity and citrusy scent followed by a balanced herbal, floral, and spicy scent in the background. The long-term average contents of α acids, β acids, cohumulone, colupulone, and hop oils are 3.62 wt%, 2.87 wt%, 24.40% rel., 45.37% rel., and 0.75 wt%, respectively.

To summarize, the varieties of a Saaz pedigree belong to fine aroma hop varieties. They are typical ones with a relatively low content of α acids that do not usually exceed the limit of 5 to 7 wt%. The relative content of cohumulone is lower than 30% rel. and, simultaneously, the total content of hop oils is about 1.0 wt%. This classification is also confirmed by genetic analysis. The Saaz and other genetically related varieties form a separate group among the aromatic hops. The category of aroma varieties comprises two subgroups of hops, the first of which belongs to "fine aroma hops", including original Saaz hops as well as new Saaz varieties such as Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz Shine.

Moreover, Saaz Comfort and Saaz Shine are promising varieties with very good resistance to fungal diseases (Pseudoperonospora humuli) and tolerance to drought. Their higher yield (2.16 and 2.21 t/ha, respectively), compared to the original Saaz (1.65 t/ha), is also significant. Even if the new varieties, Saaz Late, Saaz Brilliant, Saaz Comfort, and Saaz Shine, have no identical chemical and sensory parameters as the original Saaz, they can substitute this established standard as well. Their comparison from a brewing technology point of view is simultaneously published [27].

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Nomenclature

A, average; CV, coefficient of variation; CTAB, cetyltrimethylammonium bromide; DMX, desmethylxanthohumol; Med, median; NJ, Neighbor-Joining method; s, standard deviation; UPGMA, Unweighted Pair Group Method with Arithmetic means; X, xanthohumol.

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