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Evaluation of variability of the content of alpha and beta acids in Czech bittering hop varieties (*Humulus lupulus* L.)

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Abstract

Czech bitter varieties were evaluated between 2010 and 2022. Hop varieties displayed an average content of alpha acids between 10.24% w/w (Agnus) and 13.73% w/w (Gaia). Variability below 15%, proved good stability of alpha acids content in all tested bittering hops. All varieties showed a decrease in the content of alpha acids over a period of 13 years of hop cultivation. The highest content of beta acids was determined in Gaia (7.30% w/w). In contrast, the lowest content of beta acids was found in Rubín (3.73% w/w). The new bitter genotypes Uran and 5304 had an average alpha acid content of 11.56% w/w and 10.82% w/w, respectively. Uran showed an average beta acid content of 5.47% w/w and for the genotype 5304 it was 3.61% w/w. In terms of the alpha/beta acids ratio, the hop varieties can be divided into three groups. The first group included the varieties with an alpha/beta acids ratio below 2, i.e. Vital, Boomerang and Gaia. While Agnus and Uran displayed an alpha/beta ratio slightly above 2. The highest alpha/beta ratio above 3 was found in Rubín and the 5303 genotype.

Keywords: hop; *Humulus lupulus* L.; alpha acid content; beta acid content; variability; linear regression

1 Introduction

From the perspective of beer brewing, hop breeding efforts in the Czech Republic have primarily focused on aroma and bittering hops. The breeding of aroma hops goes back to the mid-19th century. Semš carried out the first selection in 1853. Associate professor Karel Osvald became the founder of clonal selection, which was the first new breeding method applied to hop breeding in the indigenous regional hop vegetation. He started exploring clonal selection in 1927. As a result of his long-time work, Czech hop growing gained three clones, which were named after the breeder – Osvald´s clone 31, Osvald´s clone 72 and Osvald´s clone 114 (Fric, 1992). From the end of World War II up until the 1990s, clonal selection continued to be applied as a breeding method. However, the newly developed hop varieties (Sířem, Podlešák, Blšanka, etc.) were not successfully utilized in practice. Osvald´s clones currently take up 90% of the Czech Republic´s total hop growing area (Kršková, 2022). In the 1960s, hop hybridization or cross-breeding was introduced to hop breeding. In 1994, Bor and Sládek became the first Czech registered hop varieties resulting from hybridization (Rigr et al., 1997). Additional aroma hop varieties (Premiant, Harmonie, Bohemie, Saaz Late etc.) have been registered since 1996 until recently (Nesvadba et al., 2021). When it comes to aroma hops, the aim is to reach a content of alpha bitter acids between 3 and 9% w/w and an alpha/beta ratio from 0.6 to 2.0.

Historically, hop farming around the world has focused primarily on high alpha acids content. From the 1960s, genotypes with an alpha content above 12% w/w were developed in England (Harley, 1968), Yugoslavia (Krajl, 1976), the United States (Haunold et al., 1976), Germany (Schildbach, 1985) and Japan (Mori, 1974). Hop varieties such as Galena, Nugget, Target, Magnum,

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Taurus, Columbus and Herkules were registered abroad, having an alpha content between 12 and 18%. Agnus was registered as the first Czech Republic´s bittering hop variety in 2001 (Nesvadba and Krofta, 2002), followed by Rubín in 2007, Vital in 2008, Gaia and Boomerang in 2017. The yield of Czech bitter hops is between 2.1 and 3.0 t/ha (Nesvadba et al., 2022a). In 2022, the growing area of bitter hop varieties totalled 76 ha (Kršková, 2022).

Hop resins belong to the most important substances that affect the quality of hops. Resins are divided into soft resins (alpha-bitter acids and beta-bitter acids), non-specific soft resins and hard resins. Alpha-bitter acids are represented by seven humulone analogues, beta-bitter acids by seven lupulone analogues (Verzele, 1979). The representation and content of the individual analogues is dependent on the hop variety as well as climatic and growing conditions. The content of alpha-bitter acids belongs to the major quality parameters in the boiling process (Krofta et al., 2022). During the hop boiling process, compounds are isomerized into iso-alpha-bitter acids, which are responsible for the intensity and character of bitterness, depending on the hopping dose and the quality of hops used (Basařová et al., 2021).

In recent years, hop breeding has focused on drought resistance. The starting point is to test the existing hop varieties in terms of their drought resistance. Then, genotypes with the lowest year-on-year variability are used for the development of new genetic material (Nesvadba et al., 2022b). The content of alpha and beta acids is a crucial parameter for breweries. However, the stability of this parameter needs to be taken into account as well.

2 Materials and methods

2.1 Hop varieties

Five bittering hop varieties that are currently registered in the Czech Republic were evaluated in the years 2010– 2022. They are as follows:

Agnus (registered in 2001) was gained by selection from hybrid descendants with the Sládek, Bor, Saaz, Northern Brewer and Fuggle hop varieties as well as additional breeding materials in their origin. It has a strong hoppy or even spicy aroma and a herbal scent in the background. The aroma is highly intense.

Rubín (registered in 2007) was gained by selection from the descendants of Bor and a male plant that is a multiple cross of hybrid materials (Saaz and Northern Brewer). Its aroma is spicy, floral and herbal. After having reached its technical maturity, the aroma can show traces of sulphur. The aroma is medium intense.

Vital (registered in 2008) was developed from the Agnus maternal variety and a paternal plant gained from semi-finished breeding materials. It has a hoppy or even spicy aroma with a fruity and herbal scent in the background. The aroma is highly intense.

Gaia (registered in 2017) was gained from Agnus and a male plant originating from the Yeoman hop variety from England and breeding materials of Czech and foreign hop varieties. It has a hoppy or even spicy aroma with a fruity and floral scent in the background. The aroma is medium to highly intense

Boomerang (registered in 2017) was developed by selection from hybrid descendants originating from a multiple hybridization of Agnus, Magnum and Premiant as well as semi-finished breeding materials with Saaz, Sládek, Northern Brewer and Fuggle in their origin.
Its aroma has a higher share of a spicy scent and a hoppy, woody, herbal and fruity scent in the background. The aroma is highly intense.

Since 2021, a research project entitled "*Application of new hop varieties and genotypes resistant to drought in hop growing and beer brewing*" (QK21010136) has been implemented within a program of the National Agency for Agricultural Research. The objective of the project has been to gain new hop varieties resistant to drought. In 2022, six new genotypes were included in the registration tests of the Central Institute for Supervising and Testing in Agriculture, two of which are bitter hop genotypes:

Uran is a multiple cross of hybrid materials of European and American bittering hops. This genotype was evaluated from 2012 to 2016 only. Its registration is likely in 2025.

Its aroma has a higher share of a spicy scent and is also woody with forest berries in the background. The overall aroma is highly intense.

The **5304 genotype** was gained after the hybridization of European bittering hop varieties, with the highest share of Agnus and Taurus. This genotype was evaluated from 2016 to 2022 only. Its registration is possible in 2025.

It has a hoppy aroma with a spicy and herbal scent in the background. The aroma is medium intense.

Due to the fact that these 2 genotypes are not evaluated in the same time series, they are not included in the 12 year-long statistical evaluation either. The results are for information only.

2.2 Hop growing

The evaluation was carried out in the Žatec region in the village of Stekník (latitude and longitude are described as 50.324085N; 13.523169E). The evaluated genotypes were grown under the following conditions:

The hop field is located at an altitude of 215 meters in the Žatec hop growing region and the Ohře River Basin hop growing location. The region is warm and

dry. The sum of temperatures above 10 °C amounts to 2,600–2,800 °C per year. From a pedological perspective, there are alluvial soils, which are light with colluvial and alluvial sediments. They can get dry. Soil angle – a complete plain with no signs of sheet water erosion, the land is exposed on all sides. The soil is skeletonless and over 60 cm deep.

2.3 Hop evaluation

This evaluation was part of a maternal plants assessment in maintenance breeding. Such plants are not virusfree and come from original maternal material. At least 40 plants of each hop variety are monitored in maintenance breeding and 10 mother plants are evaluated annually. Each mother plant is evaluated in terms of its morphological characteristics. Deviations from uniformity of the hop variety are monitored. Characteristics evaluated in every mother plant include hop yield, content and composition of hop resins, content and composition of hop oils, mechanical analyses of dry hop cones and aroma of hop cones.

Each plant is harvested separately. An experimental Wolf picking machine is used for hop picking. An average sample was taken from each hop variety and dried at a temperature of 56 °C to reach a humidity of 7%. The content of alpha bitter acids was determined from dry hop cones by using liquid chromatography according to EBC 7 (Krofta, 2008).

The following statistics were prepared: average (x) and standard deviation (s). Relative amount of variability is used to compare a set with different levels. Resulting variability amounts are dimensionless numbers (usually in %). This makes it possible to compare the variability of statistical characteristics differing in measure units. Coefficient of variation (CV), showing the extent of variability in %, was used for data processing. A paired t-test was applied to determine and prove the significance of difference between hop varieties. The difference of sets was determined on the basis of significance level (α), which shows the probability of difference of the tested sets (Meloun and Militký, 1994). For example, if the significance level is determined as α = 0.01, it means there is a 99% probability that the sets under review are different. Linear regression was used to evaluate a trend over a 10 year-long period. Dependence was determined by using the coefficient of determination (r^2). A centuple of the coefficient of determination shows in % to what extent the value of the alpha or beta acids content is influenced by the age of the plants.

3 Results and discussion

The Czech bittering hop varieties displayed an average content of alpha acids between 10.24% w/w (Agnus) and 13.73% w/w (Gaia), see Table 1. There is a 99% probability that Gaia reached the highest content of alpha acids compared to the other hop varieties (Table 2). Further, Vital showed a significantly higher content of alpha acids than Rubín and Agnus. Boomerang had a significantly higher content of alpha acids only when being compared to Agnus. The results prove that hop breeding has been successful in gaining new bittering hop varieties with a rising trend in alpha acids content. Based on the low variability below 15%, it can be stated that all bittering hops demonstrated a good stability of alpha acids content. An average content of all the above varieties fell within the range specified in Czech Hops Varieties (Nesvadba et al., 2022c). Trefilová et al. (2021) evaluated the content of alpha acids in Gaia and Boomerang within the collection of genetic resources in the Czech Republic between 2016 and 2020. Their results are almost identical with those achieved in our evaluation: Gaia and Boomerang had an alpha acids content amounting to 13.34% w/w and 12.06% w/w, respectively.

Table 1 *Average alpha acids content and its variability (Stekník 2010–2022)*

Parameter	Agnus	Rubín	Vital	Gaia	Boomerang
Average (% w/w)	10.24	11.33	12.26	13.73	11.83
Standard deviation	1.33	1.05	1.18	1.33	1.34
Coefficient of variation (%)	13.00	9.29	9.58	9.66	11.34

Table 2 *Significance of difference in the alpha acids content determined by using a paired t-test*

	Gaia			
Vital	0.01	Vital		
Boomerang	0.01	–	Boomerang	
Rubín	0.01	0.01	–	Rubín
Agnus	0.01	0.01	0.01	–

As specified above, the new genotypes – Uran and 5304 – are undergoing registration tests. In the period from 2012 to 2022, Uran had an average alpha acids content of 11.56% w/w and variability of 12.36%. In this period, it

showed a significantly lower alpha acids than Gaia with a 99% probability. Also, there was a 99% probability that its content was higher than that of Agnus. No statistically significant difference in the alpha acids content was determined compared to the other hop varieties. The 5304 genotype displayed an average alpha acids content of 10.82% w/w and variability of 10.13% in the period from 2016 to 2022. In the course of a 7 year-long evaluation period, it showed a significantly lower content of alpha acids than Gaia with a probability of 99%. No statistical significance of difference in the content of alpha acids was determined with respect to the other hop varieties.

The Agnus variety demonstrated a decrease in the alpha acids content over a 13 year-long period of hop cultivation (Figure 1). The reliability value of this trend was low ($r^2 = 0.27$). A centuple of the determination coefficient suggested that the length of the hop growing accounts for 27% of this decrease. The trend of year-on-year decrease in the alpha acids content was due to the y value (-0.1777x), which presents an annual decrease of 0.18% w/w. This trend corresponds to a decrease in the content of alpha acids by 1.78% w/w in 10 years. Agnus had the highest content of alpha acids in the years 2013 and 2016, namely above 12% w/w. In contrast, the lowest content of alpha acids in Agnus was determined in the years 2014, 2017 and 2022, namely below 9% w/w.

Rubín displayed an almost as identically decreasing trend as Agnus (Figure 2). The centuple of the determination coefficient showed that the length of the hop growing accounted for 24% of this decrease. The trend of year-on-year decrease in the content of alpha acids was due to the y value, which presents an annual decrease of 0.13% w/w. An alpha acid content above 12% w/w was determined in the years 2010, 2013, 2015 and 2016. Only in 2018 the content of alpha acids was below 8% w/w.

Figure 3 illustrates a small decrease in the alpha acid content of the Vital variety, namely 11.3% over a 10 year-long growing period. At the same time, it demonstrated a lower dependence on the period of growing because the centuple of the determination coefficient amounted to 14%. The highest content of alpha acids was observed in 2011 (14.02 % w/w) and the lowest in 2018 (9.97% w/w). It can be concluded that among the older bittering hop varieties (registered before 2010)

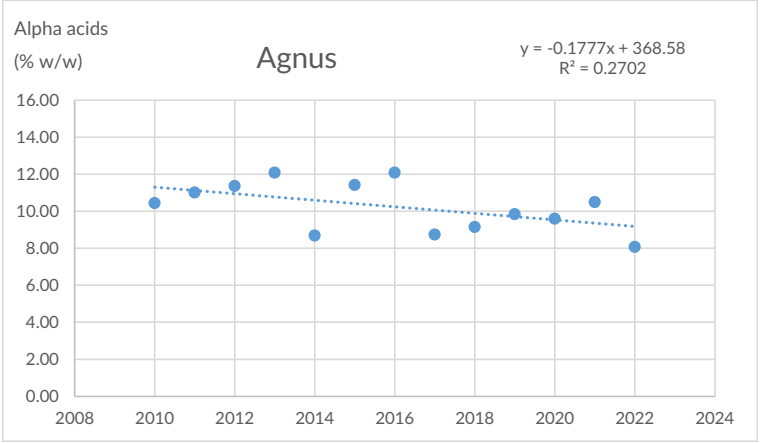

Figure 1 *Linear regression of the alpha acids content in the Agnus variety (Stekník 2010–2022)*

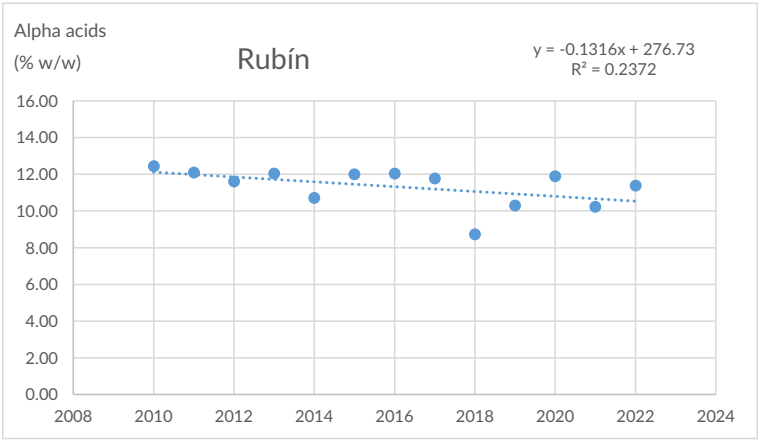

Figure 2 *Linear regression of the alpha acids content in the Rubín variety (Stekník 2010–2022)*

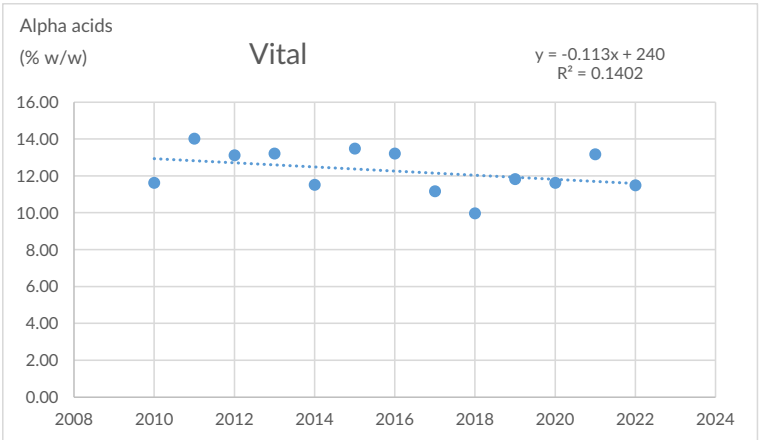

Figure 3 *Linear regression of the alpha acids content in the Vital variety (Stekník 2010–2022)*

Vital showed a better stability of alpha acids content. The resulting values of alpha acids content confirm that Vital had a content of alpha acids between 10 and 15% w/w. (Krofta et al., 2013), which was in line with the data published 10 years ago.

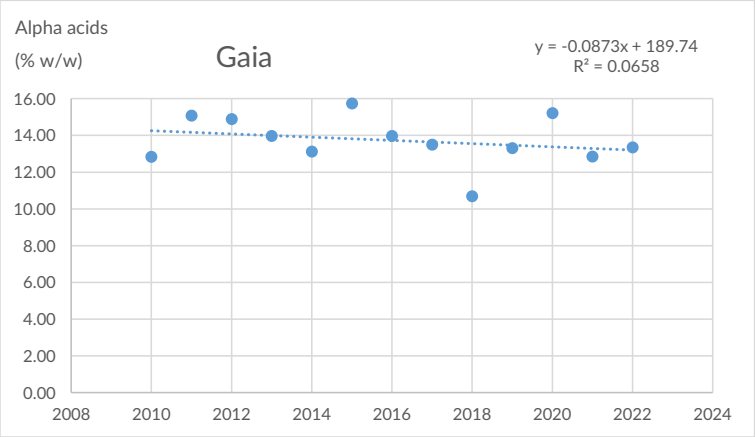

Figure 4 *Linear regression of the alpha acids content in the Gaia variety (Stekník 2010–2022)*

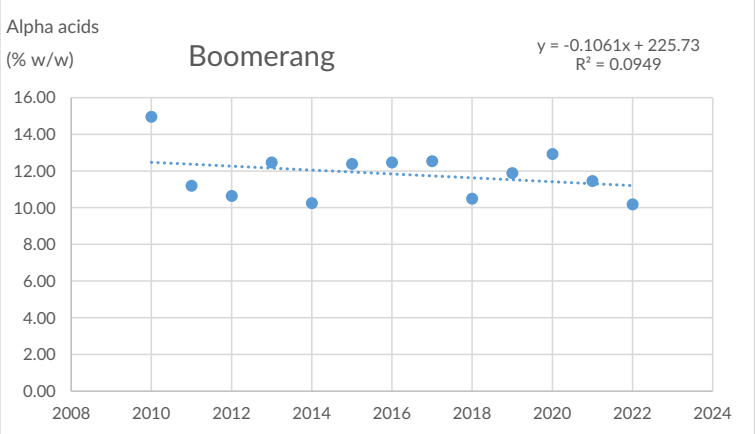

Figure 5 *Linear regression of the alpha acids content in the Boomerang variety (Stekník 2010–2022)*

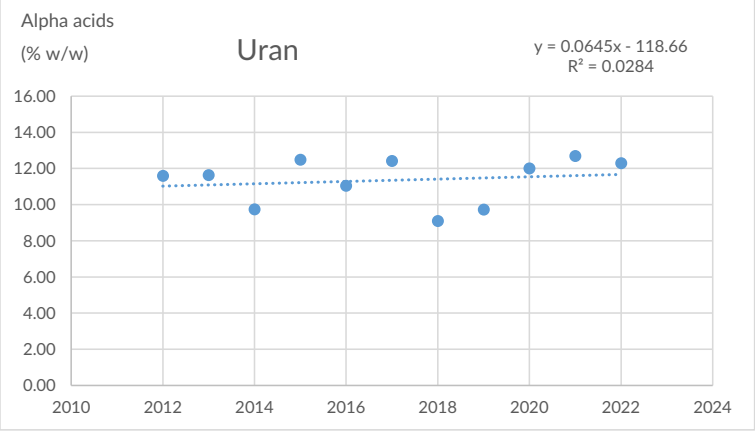

Figure 6 *Linear regression of the alpha acids content in the Uran genotype (Stekník 2010–2022)*

In 2017, a new generation of bittering hop varieties was registered, namely Gaia and Boomerang. Figures 4 and 5 illustrate that both hop varieties recorded a small year-on-year decrease in the content of alpha acids, namely 0.09% w/w (Gaia) and 0.11% w/w (Boomerang). Both hop varieties also showed a lower dependence of the decrease in alpha acids content on the growing period since the centuple of the determination coefficient is 7% for Gaia and 9% for Boomerang. The results demonstrate that hop breeding has been successful in gaining new bittering hop varieties with a higher stability in the alpha acids content over the 13 year-long growing period.

Linear regression of the alpha acids content over the growing period was also determined in the new genotypes bred for drought resistance. It is interesting that both genotypes demonstrated a trend of increasing dependence. In terms of data significance, this trend cannot be confirmed for the 5307 genotype because it is only a 7-year series. This genotype reached the lowest content of alpha acids in 2018 (8.99% w/w) and the highest in 2021 (12.48% w/w). There is an 11-year time series for Uran, which is already significant from the perspective of hop growing. Figure 6 makes it clear that the y value shows an increasing trend in alpha content, namely 0.06% per year, which corresponds to an increase of alpha acids content by 0.65% w/w over a 10-year period. The impact of the age of plants on dependence accounted for only 2.84%. This factor is important because Uran did not exhibit a decrease in alpha acids content. The figure also shows that alpha acids content was below 10% in 2014, 2018 and 2019. In 2015, 2017 and from 2020 to 2022, this parameter was above 12% w/w.

Table 3 shows that the highest content of beta acids was determined in Gaia (7.30% w/w). In contrast, the lowest content of beta acids was found in Rubín (3.73% w/w). Table 4 also shows that Gaia did not demonstrate a significant difference in the content of beta acids only when being

compared to Vital. Vital did not exhibit a significant difference in the content of beta acids only when being compared to Boomerang. In contrast, with a 99% probability Rubín reached a significantly lower content of beta acids than the other hop varieties. Agnus also had a significantly lower content of beta acids than Gaia, Vital and Boomerang. Nesvadba et al. (2020) evaluated the beta content in Czech hop varieties in the years 2010 to 2019. All bittering hop varieties had a higher average beta acids content in this period than between 2010 and 2022 – Agnus (5.33% w/w.), Rubín (3.88% w/w), Vital (7.35% w/w), Gaia (7.58% w/w) and Boomerang (6.83% w/w).

Table 3 *Average beta acids content and its variability (Stekník 2010–2022)*

Parameter	Agnus	Rubín	Vital	Gaia	Boomerang
Average (% w/w)	4.92	3.73	6.98	7.30	6.61
Standard deviation	1.31	0.43	1.23	1.73	1.12
Coefficient of variation (%)	26.64	11.49	17.60	23.62	17.01

Table 4 *Significance of difference in the alpha acids content determined by using a paired t-test*

	Gaia			
Vital	-	Vital		
Boomerang	0.1	-	Boomerang	
Agnus	0.01	0.01	0.01	Agnus
Rubín	0.01	0.01	0.01	0.01

Uran showed an average beta acids content of 5.47% w/w and variability of 14.59%. While the 5304 genotype displayed an average beta acids content of 3.61% w/w and variability of 8.53%. With a 99% probability, Uran reached a significantly lower content of beta acids than Gaia, Vital and Boomerang and with a 99% probability a significantly higher content of beta acids than Agnus and Rubín. There is the same 99% probability, the 5304 genotype had a significantly lower beta acids content than Gaia, Vital, Boomerang and Uran. No statistically significant difference was determined when Agnus and Rubín were compared.

In terms of the alpha/beta ratio, the hop varieties can be divided into three groups (Table 5). The first group included the varieties with an alpha/beta ratio below 2, i.e. Vital, Boomerang and Gaia, while the second one comprised varieties with an alpha/beta ratio slightly above 2, i.e. Agnus and Uran. The varieties of Rubín and the 5304 genotype formed the third group with the highest alpha/beta ratio, namely above 3. Rubín showed the lowest variability of the alpha/beta ratio (10.76%). In contrast, the highest variability was determined in Gaia (23.02%) and Agnus (20.36%). Agnus, the first Czech bittering hop variety, was registered with a higher content of beta acids and has an alpha/beta ratio of 2 (Nesvadba and Krofta, 2002). The results illustrated that this hop variety maintained the ratio even 20 years after its registration.

Table 5 *Average and variability of the alpha/beta acids ratio*

Parameter	Agnus	Rubín	Vital	Gaia	Boomerang	Uran	5304
Average (% w/w)	2.12	3.06	1.80	1.97	1.82	2.12	3.01
Standard deviation	0.43	0.33	0.31	0.46	0.25	0.32	0.35
Coefficient of variation (%)	20.36	10.76	17.32	23.02	13.55	15.17	11.56

Table 6 *Production of alpha acids per 1 hectare of hops*

Variety	Yield (t/ha)	Content of alpha acids (% w/w)	Production of alpha acids (kg/ha)
Agnus	1.92	10.24	196.61
Rubín	1.98	11.33	224.33
Vital	2.19	12.26	268.49
Gaia	2.24	13.73	307.55
Boomerang	1.58	11.83	186.91
Uran	2.56	11.56	302.85
5304	2.64	10.82	285.65

If we look at profitability of hop growing, the alpha acids production per hectare of a hop growing area is a very important indicator as well. Table 6 shows that only Gaia and Uran achieved a production of alpha acids above 300 kg/ha. Contrary to that, Agnus and Boomerang produced less than 200 kg of alpha acids/ha. The 5304 genotype, which is resistant to drought, also showed a high production of alpha acids (285.65 kg/ha). It needs to be noted that the average yield of Uran hops was calculated for a 10-year time series and of the 5304 genotype for a 7-year time series only.

4 Conclusion

The results achieved show that hop breeding has succeeded in gaining new bittering hop varieties with better performance parameters. The highest performance was recorded in Gaia, which was registered in 2017. The new Uran genotype also demonstrated a high performance. Another promising genotype – 5304 – needs to be mentioned as well; together with Uran they were bred with the aim to be drought resistant. Unfortunately, Boomerang was the least productive hop variety in terms of performance parameters. The results have clearly proved which varieties display a good stability in the alpha and beta acids production. This is very important both for hop growers and breweries.

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