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Old but still good – Comparison of malting and brewing characteristics of current and historical malting barley varieties

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Abstract

Historical Czech malting barley varieties Chlumecký, Stupický staročeský, Opavský Kneifel, and Diamant were tested in pilot malting and brewing tests (50 L) of 12% pale lager and compared with five Czech barley varieties recommended for the production of the beer with the protected geographical indication 'České pivo'. The grain yield of the historical varieties (6.00–7.83 t/ha) was lower compared to the current varieties (8.23–9.39 t/ha). The malts from the historical varieties had high nitrogen content (12.45–13.89%), and low extract (75.2–78.6%), proteolytic (Kolbach index 37.4–40.9%) and cytolytic modification (friability 46.2–57.7%) was also low. Apart from lower extract yield and lower beer filtration rate, the experimental malts from the historical varieties were well processable in the pilot brewery. The sensory quality of the beers was very good (overall impression 3.3–3.8 points), fully comparable to beers made from malts from current barley varieties (3.4–3.9 points). Cluster analysis clearly differentiated the sensory profile of beers of historical and current barley varieties. The historical malting barley varieties under study may find their use mainly in the production of regional microbreweries.

Keywords: malting barley varieties; old Czech barley varieties; malt; beer; sensory quality

1 Introduction

Historical varieties of malting barley are the result of long-term human effort and interaction with unique local conditions. From this perspective, historical varieties are the heritage of our ancestors, similar to architectural monuments. Until the 1870s, barley breeding in the territory of the today's Czech Republic was limited to the primitive selection of seed from better stands in more or less closed areas. This led to the development of landraces. The landraces were heterogeneous populations, i.e. sets of genotypically different but genetically related individuals of the same species. They were undemanding, drought-resistant, early, but extensive (Lekeš, 1997). In 1842, the Bavarian brewer J. Groll brewed the first golden pale lager from these varieties at the Měšťanský pivovar in Pilsen, which later, under the name Pilsner Urquell, conquered the whole world. Some areas have become famous for the quality of their barley. In Central Europe, these areas include Moravia (especially the Haná region, a fertile area along the Morava and Haná rivers) and Bohemia (especially the lowland along the river Elbe (Polabí)), where barley of excellent malting quality has been produced (Lein, 1963).

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The first variety of spring barley in the territory of the today's Czech Republic was bred by E. Proskowetz in 1884. It was named Proskowetz Hanna Pedigrée after the breeding method used. In 1926, R. Kneifel bred the Opavský variety (also called Kneifl, Kneifl barley, Kneifl barley – Opavský, Opavský Kneifel). The Opavský variety was the first representative of the so-called whole-grain barley (Lekeš, 1961; 1997). Proskowetz Hana Pedigrée and Opavský (Kneifel) were used in breeding programmes in various parts of Europe and they have been included in the pedigrees of many well-known varieties (Lein, 1963; Lekeš, 1964; 1997; Grausgruber et al., 2002). For example, the varieties Chlumecký (1902) and Stupický staročeský (1919) were developed by targeted selection from the Old Bohemian landraces.

The first attempts to cross two varieties with each other were made in the territory of the today's Czech Republic as early as 1900 (Kyas, 1921). This method began to be used on a wider scale only around 1920. Crossing of the Valtický B variety with the Starnovsky Kneifel variety gave rise to Valtický C in 1938. In the middle of the 20th century, the varieties originating in the Haná region were considered to be the best malting varieties in the world (De Clerck, 1964).

In the 19th and early 20th centuries, landraces and the first varieties that were created by targeted selection from the landraces were replaced by newer varieties based on better yield or other agronomic characteristics. However, this replacement of varieties did not take into account the characteristics associated with the malting quality and sensory quality of the beer.

Diamant (1965) is much younger than the above varieties. However, it is interesting for the method that was used at the beginning of its breeding. The Diamant variety was bred from a radio-mutant of the Valtický variety and is now in the pedigree of several dozen varieties worldwide.

In the Czech Republic, we strive to preserve the sensory character of beer as the Czech national beverage (Psota, 2003; Kosař et al., 2004). For this reason, an application for awarding the protected geographical indication (PGI) 'České pivo' was submitted in 2008 (European Committee of the Regions, 2008). The varieties recommended for the production of beer with the PGI 'České pivo' have a lower level of proteolytic and cytolytic modification and a lower level of fermentation causing the presence of residual extract.

It is not known much about the original landraces or the first varieties created by targeted selection. We often do not know their economic characteristics (yield, disease resistance, lodging, etc.). We do not know how they may react to the malting and brewing methods used today, or whether they have the specific quality characteristics sought today. Historical barley varieties could, with the help of modern technology, bring back from the past beers with an interesting colour, a delicious taste and other characteristics that would be attractive both for producers and consumers.

The oldest historical varieties can be a valuable source of alleles for coping with a changing environment (Nevo et al., 2012). Thanks to the great genetic diversity, landraces and the first purposefully bred varieties of barley can be a suitable model for researching the adaptation of barley to climate change.

In addition to the specific raw materials, malt and hops, and their minimum amounts in the brew, the application for the protected geographical indication 'České pivo' also prescribes the technological framework for production; this includes, in particular, the decoction mashing process, two-phase fermentation with lager yeast strains and a ban on the use of enzyme preparations (European Committee of the Regions, 2008). There is no doubt that the resulting sensory quality of beer is a combination of the brewing raw materials used and the technological process of beer production (Basařová et al., 2017). This fact is widely acknowledged, and most recently, the nano-scale brewing tests have provided clear evidence that barley genotype contributes significantly to many sensory descriptors (Herb et al., 2017).

The rapid increase in the number of craft breweries in the US and subsequently in Europe came about as a reaction to the uniform mass production of large brewing companies, with small businesses coming up with a range of non-conformist, tastefully interesting, sometimes even sensory-shocking beers. The boom of dry-hopping with the arrival of microbreweries on the market is well known.

There are about 500 microbreweries in the Czech Republic and about 100 in Slovakia. The expansion of microbreweries in the Central European region in the last decade has also brought new challenges in terms of domestic or regional raw materials. One of the possibilities is the use of historical varieties of malting barley.

The aim of the study was to compare malts and beers made from four historical Czech barley varieties with current malts and beers made from varieties recommended for the production of beer with the protected geographical indication 'České pivo' and to describe the sensory profile of beers made from historical barley varieties.

2 Materials and methods

2.1 Barley and malt

The seed of the historical varieties Chlumecký, Stupický staročeský, Opavský Kneifel, and Diamant was obtained from the Collection of Genetic Resources of Spring Barley administered by the Agricultural Research Institute in Kroměříž. In 2017, the historical and current varieties (Bojos, Francin, Laudis 550, Malz and Petrus) were sown on the land of Ditana, s.r.o. in the site of Velká Bystřice, near Olomouc (Haná region). The land is at an altitude of 270 m in the sugar beet production region, the soil is clayey-loam. The long-term average (1971–2000) sum of precipitation is 502 mm and a long-term average temperature is 8.7 °C. In 2017, the annual rainfall sum was 663.5 mm and the average annual temperature was 10 °C. The pre-crop was sugar beet with ploughed-in leaves. Before sowing (15 March 2017), calcium ammonium nitrate was applied to the field. Sowing took place on 27 March 2017 and the sowing rate was 3.5 million germinated grains per hectare. The plants of the historical varieties are mostly tall and lodge heavily. To shorten the stem and increase resistance to lodging, they were treated with growth regulators (active ingredients chlormequat-chloride and trinexapac-ethyl). In addition, herbicides against dicot weed, fungicides against fusarium and insecticides against Oulema beetles and aphids were applied. Harvesting took place on 31 July 2017.

The historical varieties were compared with the following current varieties: Bojos (Psota et al., 2005), Francin (Psota et al., 2014), Laudis 550 (Psota et al., 2013), Malz (Psota and Jurečka, 2002), and Petrus (Psota et al., 2013) recommended for the production of beer with the PGI 'České pivo' (European Committee of the Regions, 2008).

Malts were prepared in a laboratory malting device (KVM Uničov, Czech Republic) according to the Mitteleuropäische Brautechnische Analysenkommission (MEBAK, 2018) methodology (R-110.00.008 Micro-malting): 72 hours steeping at 14 °C with CO_2 exhaustion, steeping periods on 1st day 5 hours, 2nd day 4 hours and 3rd day 3 hours. Germination time was 72 hours at 14 °C. Pre-drying took 12 hours at 55 °C and kilning 4 hours at 80 °C.

2.2 Brewing trials

The pilot brewery tests with the experimental malts were carried out on a 50-litre line (Pacovské strojírny, Pacov, Czech Republic) in the pilot brewery of the Research Institute of Brewing and Malting (RIBM). The malt was ground using a two-roller mill. The ratio of malt grist to water in mashing-in was 1:3.7. The sweet wort preparation of all-malt 12% brews was carried out according to the single-decoction protocol as follows: mashing-in at 37 °C (10 min); heating to 52 °C (0.8 °C/min), rest (20 min); heating to 63 °C (0.8 °C/min), saccharification rest (20 min); transfer of thick mash, heating to 72 °C (0.8 °C/min), saccharification rest (15 min); heating to 100 °C (1.3 °C/min), mash boiling (15 min), pumping; mashing-off at 77 °C. The mash solids were separated from the sweet wort using a lauter tun, while the volume of the sweet wort remained constant. The lautering rate was measured as the average wort flow rate over the entire operation, lautering and sparging. Hopping doses were 50% (CO_2 extract) at the beginning, 35% (Saaz pellets 90) after 30 minutes, and 15% (Saaz pellets 90) 10 minutes before the end of the 70-minute boiling process.

After separation of the hot trub in the whirlpool, the wort was cooled down to the fermentation temperature of 10 °C and aerated at 8 ± 0.5 mg/L of dissolved oxygen. Primary fermentation was performed in cylindrical-conical tanks at an approximate pitching rate of 17×10^6 cells/mL of yeast strain No. 95 of the RIBM Collection. The green beer was chilled down and transferred to "lager" CCTs. Maturation took three weeks at 1–2 °C.

The beers were filtered using depth filtration plates composed of cellulose, kieselguhr and perlite, then packaged in 500 mL glass bottles, and finally pasteurized in an immersion pasteurizer. The filtration rate was measured as the average beer flow rate over the filtration of 30 litres.

2.3 Barley, malt and beer analysis

The barley malts were analysed according to the European Brewery Convention (EBC) Analysis Committee methodology (Analytica-EBC, 2010), the following methods were used: 3.2 Moisture Content of Barley, 3.3.1 – Total Nitrogen of Barley: Kjeldahl Method, 4.2 Moisture Content of Malt, 4.3.1 – Total Nitrogen of Malt: Kjeldahl Method (IM), 4.5.1 Extract of Malt: Congress Mash, 4.9.1 – Soluble Nitrogen of Malt: Kjeldahl Method, 4.10 Free Amino Nitrogen of Malt by Spectrophotometry, 4.11.1 Fermentability, Final Attenuation of Laboratory Wort from Malt, 4.12.2 Diastatic Power of Malt, 4.15 Friability, Glassy Corns and Unmodified Grains of Malt by Friabilimeter, 4.16.2 High Molecular Weight β-glucan Content of Malt: Fluorimetric Method, 8.7 – Fermentable Carbohydrates in Wort by HPLC (IM), 8.17 – pH of Wort, 9.11 Total Polyphenols in Beer by Spectrophotometry, 9.29 Haze in Beer: Calibration of Hazemeters. Some analyses were performed according to Mitteleuropäische Brautechnische Analysenkommission (MEBAK, 2011; 2018) methodology using the following methods: R-205.05.730 Appearance (MEBAK, 2018), 3.1.4.11 Hartong-Kretschmer Mash Method, VZ 45 °C (MEBAK, 2011). Arabinoxylans (Pentosans) were measured by Douglas Method (Douglas, 1981).

Beer analyses were carried out according to the Analytica-EBC (2010), the following methods were used – 9.4 Original, Real and Apparent Extract and Original Gravity of Beer, 9.2.6 Alcohol in Beer by Near Infrared Spectroscopy, 9.35 pH of Beer, 9.7 Final Attenuation of Beer, 9.6 Colour of Beer: Spectrophotometric Method, 9.10.1 Free Amino Nitrogen in Beer by Spectrophotometry, 9.8 Bitterness of Beer, 9.11 Total Polyphenols in Beer by Spectrophotometry. Foam Stability was determined by the MEBAK 2.18.2 method (MEBAK, 2013). Sensory analysis was carried out using the EBC method 13.10 Sensory Analysis: Description Analysis (Analytica-EBC, 2010) by a RIBM panel of trained assessors. Basic descriptors – carbonation, palate fullness, bitterness, astringency, sourness and sweetness and malt-derived descriptors, i.e. malty, perfume, caramel, grainy, syrupy and after wort flavour (ascending scale 0–5; imperceptible–very strong) were taken into the account. Bitterness was assessed by maximum sensation (at 15 seconds after drinking) and character of bitterness (gentle to harsh-clinging). Overall impression, the overall assessment of the sample, with respect to the appropriateness of the all attributes present, including off-flavours, their intensities, and the unidentifiable background flavour, was rated on a descending scale (1 – excellent to 9 – inappropriate). The data was processed by a cluster analysis.

3 Results and discussion

In this study, we assessed the processability of grain from historical barley varieties in the malting and brewing process and the sensory quality of the resulting beer. The preferred beer style in the Czech Republic is the traditional Czech (Pilsner) lager. Its raw material base was, and still is, malts from domestic barley varieties and traditional Saaz hops. The results of the malt and beer analyses were evaluated with regard to the specific requirements for the quality parameters of malts and beers described in the application for the PGI 'České pivo'.

3.1 Barley and malt

In the historical varieties studied, leaf diseases (especially net blotch of barley) occurred heavily. The yield of the historical varieties ranged from 6.00 to 7.83 t/ha. The yield of the current varieties ranged from 8.23 to 9.39 t/ha. The historical varieties thus had an average yield 1.2 t/ha lower than the current varieties (Table 1).

The results of the malt analyses showed the differences in the individual characters across the varieties (Table 2). The seed samples of the nine spring barley varieties contained significantly different amounts of nitrogenous substances. The nitrogen content of barley grain influences the quality of malt and wort; this was also evident in the set of varieties studied.

The samples of Malz, Bojos, and Laudis 550 with the optimum grain nitrogen content gave malt with satisfactory to optimum extract content (82.5–83.1%). Diastatic power was at optimum level in these and all other samples. Quality of the wort, as determined by the apparent attenuation achieved, was low (79.6%) for the Bojos sample. Quality of the wort was good to optimum for Laudis 550 (80.8%) and Malz (81.5%) samples. All three samples fulfilled the requirement for this attribute set out in the application for the PGI 'České pivo'. Only the sample of the Bojos variety met the requirement for the level of proteolytic modification specified in the PGI 'České pivo' and showed a Kolbach index value of less than 42%. The samples of Laudis 550 and Malz significantly exceeded this requirement.

Cytolytic modification was at optimum levels for the Bojos and Malz samples. In case of Laudis 550, cell wall degradation was at a satisfactory level (83%), but the ß-glucan content of the wort was high (197 mg/L). All three varieties met the requirement for cell wall degradation set out in the application for the PGI 'České pivo'. The samples of the Francin and Petrus varieties showed high nitrogen content in the barley grain; this corresponded to the low extract content in the wort made from these samples (81.2 and 81.4%). The samples of Francin and Petrus met the requirement for the extract content of the PGI 'České pivo'. The diastatic power of these samples

Table 1 *Grain yield of the tested barley varieties*

Variety	Accession number*	Registration	Origin	Yield t.ha^{-1}
Chlumecky	03C 0600023	1902	AHE	6.05
Stupicky Starocesky	03C 0600021	1919	CSK	7.46
Opavsky Kneifel	03C 0600005	1926	CSK	6.00
Diamant	03C 0600166	1965	CSK	7.83
Malz	03C 0602668	2002	CZE	8.23
Bojos	03C 0602742	2005	CZE	9.39
Laudis 550	03C 0603050	2013	CZE	9.09
Petrus	03C 0603053	2013	CZE	9.33
Francin	03C 0603105	2014	CZE	9.12

* Genetic Resources in the Czech Republic – GRIN Czech (https://grinczech.vurv.cz/gringlobal/search.aspx)
AHE – Austro-Hungarian Empire; CSK – Czechoslovakia; CZE – Czech Republic

Table 2 *Results of experimental malt analysis*

Parameter	Unit	Bojos	Francin	Laudis 550	Malz	Petrus	Diamant	Chlumecký	Opavský Kneifel	Stupický staročeský
Accession number*	–	03C0602742	03C0603105	03C0603050	03C0602668	03C0603053	03C0600166	03C0600023	03C0600005	03C0600021
Registration	–	2005	2014	2013	2002	2013	1965	1902	1926	1919
Origin	–	CZE	CZE	CZE	CZE	CZE	CSK	AHE	CSK	CSK
Moisture content of barley	%	13.9	12.3	12.9	13.3	11.6	11.3	11,0	10.9	11.3
Protein content of barley, Kjeldahl method (factor 6.25)	%	11.0	12.0	11.6	10.7	12.9	13.8	14.9	14.7	13.4
Moisture content of malt	%	4.50	4.73	4.64	4.68	4.94	4.69	4.66	4.35	4.35
Appearance (clarity) of wort	–	clear	clear	clear	clear	clear	weakly opalizing	weakly opalizing	weakly opalizing	clear
Haze of wort (90°)	EBC	1.37	0.85	0.75	0.49	0.68	2.60	3.50	3.08	1.64
Haze of wort (12°)	EBC	1.38	0.79	0.77	0.53	0.54	2.93	4.28	3.00	1.88
Saccharification time	min	10	10	10	10	10	10	10	10	10
Viscosity of laboratory wort	mPa.s	1.488	1.497	1.513	1.491	1.481	1.546	1.705	1.685	1.571
pH of wort	–	5.99	5.92	5.94	5.93	5.90	5.89	5.89	5.86	5.93
Apparent final attenuation	%	79.6	79.3	80.8	81.5	82.4	78.6	75.3	76.4	81.3
Diastatic power	WK	333	356	327	297	445	384	306	194	410
Free amino nitrogen	mg/L	155	173	197	184	219	227	233	223	185
Total polyphenols in wort	mg/L	57	58	54	67	69	47	45	49	48
Extract of malt, congress mash	%	82.7	81.2	82.5	83.1	81.4	78.6	76.3	75.2	77.5
VZ 45 °C	%	34.1	40.3	41.5	38.7	46.2	38.8	38.2	38.9	37.2
Colour of malt, visual method	EBC	2.5	2.9	2.8	2.7	2.9	3.0	3.0	2.9	2.6
Protein content of malt (factor 6.25)	%	10.59	11.51	10.34	10.11	11.95	12.86	13.79	13.89	12.45
Total nitrogen of malt, Kjeldahl method	%	1.69	1.84	1.65	1.62	1.91	2.06	2.21	2.22	1.99
Kolbach index	%	40.0	41.2	47.2	46.2	45.2	40.9	39.8	38.0	37.4
Soluble protein of malt, Kjeldahl method	%	4.2	4.7	4.9	4.7	5.4	5.3	5.5	5.3	4.7
Soluble nitrogen of wort, Kjeldahl method	mg/100g	676	758	778	749	863	843	879	844	745
Soluble nitrogen of wort, Kjeldahl method	mg/L	755	846	869	835	960	942	984	950	837
β-Glucan content of malt, SFA	mg/L	109	153	197	120	112	284	522	493	291
Arabonoxylans in wort	mg/L	574	463	592	541	582	700	832	693	794
Friability	%	86.6	77.1	83.1	91.2	80.0	61.8	48.8	46.2	57.7
Homogeneity (by friabilimeter)	%	99.6	98.2	99.3	99.8	98.7	90.2	79.5	72.0	90.1
Partly unmodified grains	%	0.4	1.8	0.7	0.2	1.3	9.8	20.5	28	9.9
Glassy corns	%	0.2	0.5	0.0	0.0	0.1	0.5	0.5	0.3	0.1

* Genetic Resources in the Czech Republic – GRIN Czech (https://grinczech.vurv.cz/gringlobal/search.aspx)
AHE – Austro-Ungarian Empire; CSK – Czechoslovakia; CZE – Czech Republic

was at the optimum level. The quality of the wort was very low in the samples studied. Both varieties fulfilled the requirement for an apparent final attenuation set out in the application for the PGI 'České pivo'.

The sample of the variety Francin met the requirement for Kolbach index set out in the requirements for the PGI 'České pivo'.

The samples of Francin and Petrus showed slow degradation of cell walls, but satisfactory to optimum beta-glucan content in the wort. The Francin and Petrus samples met the requirements for the level of friability (cell wall degradation) set out in the application for the PGI 'České pivo'. All four samples of the historical varieties had high nitrogen content in the barley grain. Historical spring barley varieties also showed high nitrogen content in barley grain in previous experiments (Marečková et al., 2010; 2011). The high content of nitrogenous substances in the grain of the samples studied significantly affected the extract content in the malt, which was very low and ranged from 75.2 to 78.3%. The diastatic power of the samples of the varieties Diamant, Chlumecký, and Stupický staročeský was at an optimal level. Only the sample of Opavský Kneifel had a very low diastatic power (194 WK un.). The quality of the sweet wort (apparent final attenuation) was very low (75.3–78.6%) in the samples of the historical varieties. Only Stupický staročeský showed a satisfactory degree of apparent attenuation of 81.3%.

Proteolytic modification was very low in the historical varieties studied, except for the sample of the variety Diamant, which had Kolbach index of 40.9%.

The degradation of cell walls was very slow in all the historical varieties and the ß-glucan content of the wort was high in these samples. Only the samples of the varieties Diamant and Stupický staročeský had the ß-glucan content in wort below 300 mg/L.

3.2 Processability of malts

The assessment of the malt processability in the brewing process is important both from the process and economic point of view as the extension of time or higher consumption of auxiliary materials during mashing, lautering, fermentation, and beer filtration has a direct impact on the production and economy of the brewery. Furthermore, the processability of the malt affects beer quality parameters, especially its organoleptic properties.

Mash saccharification. The time of mash saccharification was in the range of 11–16 minutes for the samples of Diamant, Stupický staročeský, Bojos, Petrus, and Chlumecký varieties, while higher values in the range of 20–25 minutes were recorded for mash from the malts made from the varieties Malz, Opavský, Kneifel, Francin, and Laudis 550. The low diastatic power of the malt made from the Opavský Kneifel variety did not significantly affect the mash saccharification time in the experimental brew (Table 3).

Wort lautering. The rate of wort lautering and sparging is a monitored technological parameter of the malt processability in the brewhouse. The average flow rate throughout the whole operation was assessed. The highest lautering rate was found for the brew with malt from the Francin variety (1.28 L/min), followed by the brew with malt of the Malz variety (1.00 L/min). The lowest values were recorded for brews with malts from Bojos (0.65 L/min) and Laudis 550 (0.59 L/min).

The lautering rate for brews with malts made from historical varieties was in the range from 0.90 (Diamant) to 0.77 (Stupický staročeský) (Table 3). The negative effect on lautering has been attributed to non-starch polysaccharides, beta-glucans and arabinoxylans, as well as substances of protein and fatty acids nature (Ford and Evans, 2001; Benismail et al., 2003; Jin et al., 2004). The lautering rate of the wort of the experimental brews did not depend on the content of proteins, beta-glucans and pentosans in the malt. Even malts from the historical varieties with high content of these substances caused by low proteolytic and cytolytic modification of the grain did not show problems with lautering in the pilot 50-litre brewhouse. Lautering rate was not demonstrably related to non-starch polysaccharides, although some malts had beta-glucan concentrations well above the reported limit of 200 mg/L (Prokeš, 2000). But as the beta-glucan content during the brewing process varies according to the malt processing technol-

Table 3 *Selected process parameters of experimental brews*

Parameter	Unit	Bojos	Francin	Laudis 550	Malz	Petrus	Diamant	Chlumecký	Opavský Kneifel	Stupický staročeský
Mash saccharification time	min	15	25	25	20	15	11	16	21	14
Wort lautering rate	L/min	0.65	1.28	0.59	1.00	0.85	0.90	0.88	0.82	0.77
Free amino nitrogen (wort)	mg/L	244	223	228	222	193	304	292	236	189
Beer filtration – rate	L/min	2.50	1.67	1.67	2.00	0.86	1.50	1.50	1.52	1.20
Beer filtration – haze	EBC	0.30	0.27	0.24	0.27	0.31	0.19	0.45	0.17	0.18

ogy used, the correlation between the malt and beer is small (Erdney et al.,1998). The low mash-in temperature and the decoction mashing reduce beta-glucan content in the wort (Sacher et al., 2016; Basařová et al., 2017).

Free amino nitrogen (FAN) in wort is monitored for two reasons. An insufficient amount of amino acids can result in insufficient multiplication of yeast cells at the beginning of fermentation, on the other hand, high amino acid values negatively affect the sensory stability of beer due to the formation of Strecker aldehydes (Vanderhaegen, 2006). For 12% Czech lager, the optimum FAN value of 210–230 mg/L is recommended (Ťopka, 2000). High values were measured for the wort from Diamant and Chlumecký malts (304 and 292 mg/L), on the contrary, a good value, lower than expected from malt analysis, was found for Opavský Kneifel (236 mg/L). The FAN value of Chlumecký staročeský was below the recommended range (189 mg/L) (Table 3). Good values were measured in the worts made from the currently cultivated varieties Francin, Laudis 550, and Malz. For Bojos malt, the FAN value was unfavourably high (244 mg/L) and significantly higher than expected from malt analysis (155 mg/L), for Petrus malt the FAN was below the recommended range. The amount of FAN in the wort is affected by the protein content of barley, the activity of proteolytic enzymes in the malt and the mashing method (Basařová et al., 2017). The combination of these factors probably caused the anomalous results in the two varieties, the correlation between FAN of malt and wort was low (r=0.421).

The extract yield in the wort in the trial brews corresponded to the extract determined in the malt (r=0.903). It was therefore confirmed that the malts from the samples of historical varieties had a lower extract yield and even the single-mash decoction mashing procedure used did not improve the extractability of these slightly modified malts with a high proportion of partially glassy grains (Table 1). It is known that decoction mashing with a low mashing-in temperature (20–35 °C) and a rest of the mash at 52 °C promotes proteolysis and degradation of non-starch polysaccharides. By boiling the mash, the unmodified parts of the barley grain become accessible to the action of malt enzymes, resulting in an increase in the extract yield (Basařová et al., 2017).

Beer fermentation and filtration. During the fermentation and maturation of beers, no failures were noted that could be related to the chemical composition of the worts prepared from historical varieties. The filterability of the beers was assessed by the average flow rate when filtering 30 L of beer and the clarity of the filtrate. The filtration rate of the beer was highest for Bojos malt (2.50 L/min) and then Malz (2.0 L/min), followed by Francin, and Laudis 550 varieties (1.67 L/min), and the lowest values were measured for beers from historical varieties. The filtration rate of the beers from Diamant, Chlumecký and Opavský Kneifel malts was 1.50 L/min, and the lowest value was 1.20 L/min for Stupický staročeský. The clarity of the filtered beers was, with the exception of beer from the Chlumecký variety (0.45 EBC), up to 0.3 EBC, beers from the old varieties Diamant, Opavský Kneifel, and Stupický staročeský had excellent clarity up to 0.2 EBC (Table 3).

The experimental malts from the historical varieties, except for poorer filterability of the beer, did not show any failure during malt processing in a pilot brewery. Further, the malts were prepared by a uniform malting process, deeper saccharolytic, cytolytic and proteolytic modification can probably be achieved by adjusting the process parameters. For example, beta-glucan degradation can be successfully set by prolonged germination (Mikyška et al., 2002), because malting barley varieties have not been bred for low beta-glucan content in the past. A weakness can be the high protein content of the grain, which is typical of historical varieties. In addition to filterability, this can damage colloidal stability in case of low protein degradation during malting and mashing, or reduce sensory stability due to too high FAN concentration in beer.

3.3 Physicochemical profile of beers

The results of the brewing trials were discussed according to the basic quality criteria with regard to the production of Czech (Pilsner) pale lager beers. The optimal values are: colour of 8–16 EBC, the difference between apparent attenuation and apparent final attenuation 1.0–9.0%, bitterness 20–45 IBU, pH 4.1–4.8 and total polyphenols concentration 130–230 mg/L.

The set of physicochemical parameters of beer: attenuation, alcohol content, colour, foaming and others give direct and indirect information about the organoleptic properties of the final product. The results are summarized in Table 4.

Attenuation. The apparent attenuation of beers was in the range from 75.7% (Laudis 550) to 85% (Petrus). The attenuation of beers from Diamant and Chlumecký malts (77.3 and 76.7%) was lower than that of Opavský and Stupický staročeský beers (80.4 and 83.1%). The final attenuation of the experimental beers was, with the exception of Francin and Laudis 550 malts, higher by 3.3% (Bojos) to 9.9% (Petrus), compared to the final attenuation of the laboratory wort (Tables 2 and 4). A clear increase in values (4.2–6.4%) was recorded for all historical varieties which provided low modified malts.

The decoction process can increase the attenuation limit compared to infusion mashing (Basařová et al., 2017). The difference between the apparent and final attenuation of beers was between 2.4% (Opavský Kneifel) and 7.3% (Petrus). For malts from historical varieties, this parameter was within 3%, except for Diamant (6%). This parameter is monitored for beers marked with the PGI 'České pivo', lower attenuation, higher content of unfermented extract is one of the reasons for the higher fullness of taste of Czech beer (Basařová et al., 2017).

Colour of the beer is one of the key attributes perceived by the consumer. The colour of the experimental beers ranged from 7.1 EBC (Stupický staročeský) to 9.6 EBC (Petrus). The colour of beers made from Diamant and Chlumecký (8.9 and 9.2 EBC) was higher than that of beers from Opavský Kneifel and Stupický staročeský malts (7.3 and 7.1 EBC). The colour of beer is formed by thermal and oxidation products arising during fermentation, mashing and wort boiling, these are mainly products of the Maillard reaction of sugars with amino acids and also polyphenolic substances (Basařová et al., 2017). The colour of beers correlated at P=0.05 with the content of total polyphenols (r=0.67), the relationship with FAN in the wort was inconclusive (r=0.30). Some of the beers did not meet the PGI requirement for this parameter, with colour ranging from 8 to 16 EBC, but in our experience, the colour of the beers in the experimental brewery is usually lower than in similar commercial beers.

Foaming ability and foam stability are among the key attributes of lager beers. It is generally known that proteins and glycoproteins are foaming agents, while bitter hop substances are foam stabilizers. Surface tension and hence foam stability is reduced by lipids, fatty acids, higher alcohols and esters. The stability of the foam of a certain beer is thus a result of factors with favourable and negative effects, i.e. substances contained in beer (Lusk et al., 1995; Segawa et al., 2002; He et al., 2006; Šavel and Brož, 2006). The head retention (foam stability) evaluated by the NIBEM method was in the range of Σ = 200 to 312 s/30 mm). The range for well-foaming beers is Σ = 220 to 250 s/30 mm, beers with excellent foaming show values above Σ = 250 s/30 mm (MEBAK, 2011). Beer from Petrus malt with high proteolytic modification (Kolbach index = 45.2) had a low foaming value (200 s/30 mm). The foaming values of the other beers were in the category of excellent foaming, so beers from Bojos and Laudis 550 malts had foaming at the level of about 250 s/30 mm, other beers had values from about 280 to 300 s/30 mm, and the beer from the Diamant variety had the highest foam stability (312 s/30 mm).

Total polyphenols. The high total polyphenol content is one of the characteristics of Czech lager. Approximately 70% of polyphenols in beer come from malt, 30% come from hops (Basařová et al., 2017). Polyphenols in barley grains and malt are bound in cell structures together with polysaccharides and proteins. They are found in the cell walls of both the endosperm and especially in the aleurone layer and the husks of malt grain, i.e. in the pericarp, testa and lemma (Siebert, 2006). The total polyphenols in beers brewed from Bojos, Francin, Chlumecký, Opavský Kneifel, and Stupický staročeský malts ranged from approximately 155 to 170 mg/L; the values in beers brewed from Laudis 550, Malz and Diamant malts ranged from 195 to 213 mg/L; the highest value was observed in beer brewed from highly proteolytic Petrus malt (230 mg/L). All the beers were within the range of the values specified in

Table 4 *Physicochemical profile of experimental beers*

Parameter	Unit	Bojos	Francin	Laudis 550	Malz	Petrus	Diamant	Chlumecký	Opavský Kneifel	Stupický staročeský
Apparent extract	% w/w	2.72	2.84	3.16	2.81	1.90	2.91	2.83	2.20	1.99
Real extract	% w/w	4.70	4.73	5.05	4.75	3.97	4.81	4.62	3.95	3.88
Alcohol by volume	% v	5.23	5.23	5.27	5.41	5.73	5.28	4.95	4.79	5.20
Alcohol by weight	% w/w	4.32	4.09	4.12	4.23	4.49	4.13	3.87	3.76	4.08
Apparent attenuation	%	79.2	77.5	75.7	81.3	85.0	77.3	76.7	80.4	83.1
Real attenuation	%	63.9	62.6	61.1	65.6	68.6	62.4	61.9	64.9	67.1
Final attenuation	%	82.9	80.6	79.8	88.0	92.3	83.3	79.5	82.8	85.8
Final/apparent attenuation difference	%	3.7	3.1	4.1	6.7	7.3	6.0	2.8	2.4	2.7
Original extract	% w/w	13.04	12.63	12.99	12.91	12.65	12.78	12.12	11.26	11.79
pH		4.62	4.80	4.76	4.64	4.62	4.76	4.87	4.62	4.64
Colour	EBC	8.8	7.8	8.9	8.9	9.6	8.9	9.2	7.3	7.1
Bitterness	IBU	31	30	29	31	30	27	28	27	28
Foam stability (NIBEM)	s/30 mm	249	298	256	277	200	312	282	290	280
Total polyphenols	mg/L	160	161	201	213	230	195	167	154	168
Clarity	EBC	0.30	0.27	0.24	0.27	0.31	0.19	0.45	0.17	0.18

the application for the PGI 'České pivo' (130–230 mg/L). The value of total polyphenols in malt (laboratory wort) did not correlate with the values in decoction sweet wort (r=0.485), but there was a close relationship between decoction sweet wort and wort (r=0.978) and also between decoction sweet wort and beer (r=0.905). The concentration of polyphenols in sweet wort and subsequently in beer is strongly influenced by the mashing technology, the polyphenols in grain bound to proteins and non-starch carbohydrates are released by the action of malt proteases during germination and mashing (Zhao and Zhao, 2012). Thus, their concentration in the wort depends on the malt modification, and intensity of mashing and the decoction procedure significantly increases their concentration in the beer (Mikyška et al., 2022).

3.3 Sensory profile of beers

The palatefullness is a marker of Czech pale beers, especially lagers. The factors influencing the sensory perception of the palatefullness of beer are not fully understood; it is generally believed that higher wort extract and lower attenuation, i.e. higher viscosity and unfermented extract, dextrins, sugars and proteins in beer have a beneficial effect (Esslinger, 2009). A significant role is attributed to proteins with a molecular weight greater than 10 kDa (Langstaff and Lewis, 1993). Fullness of the beers was rated from 2.5 (Francin) to 3.1 (Diamant), with most varieties ranging from 2.7–3.0 (Table 5).

Bitterness was at a medium level and was balanced (3.1–3.4 points), bitterness after 40 seconds was 1.7 to 2.3 points. The beer from Petrus malt had a slightly less pleasant character of bitterness compared to the other varieties (3.0 points versus 2.5–2.8 points).

Astringency was very weak to weak (1.1–1.6 points), with slightly higher values found in beers made from Laudis 550 and Stupický staročeský malts (1.5 and 1.6 points) and the lowest values (1.1–1.2 points) found for Bojos, Malz and Petrus beers. The astringency of beer is attributed to certain polyphenol substances from malt (Narziss, 1998), hops (Almaguer et al., 2014) and hordatins, i.e. alkaloids derived from malt (Kageyama et al., 2011).

Sweetness was rated in the range from 1.2 points (Bojos) to 2.3 points (Petrus), for most beers it was from 1.4–1.8 points.

The malty flavour was the lowest in Bojos (0.4 points) and the highest in Petrus (2.3 points), mostly it was 0.7–1.1 points, i.e. very weak. The perfume flavour of beers from the Chlumecký and Opavský Kneifel varieties was slightly higher (1.5 and 1.3 points) compared to the other varieties (0.4–1.2 points). The grainy flavour of beers from the his-

Table 5 *Sensory evaluation of experimental beers*

Attribute	Bojos	Francin	Laudis 550	Malz	Petrus	Diamant	Chlumecký	Opavský Kneifel	Stupický staročeský
Carbonation	2.3	2.1	2.2	2.7	2.1	3.1	3.2	2.1	2.3
Palate fullness	2.8	2.5	2.6	2.9	3.0	3.1	3.0	2.9	2.7
Bitterness	2.2	2.0	2.0	2.2	2.0	1.9	2.0	1.9	2.0
Bitterness – culmination	3.2	3.3	3.1	3.2	3.4	3.3	3.3	3.2	3.3
Bitterness-lingering	2.0	2.2	1.7	2.1	1.8	2.0	2.1	2.3	2.0
Bitterness-character	2.7	2.8	2.5	2.7	3.0	2.6	2.7	2.8	2.7
Astringency	1.2	1.3	1.5	1.1	1.2	1.4	1.3	1.4	1.6
Sweetness	1.2	1.8	1.8	1.5	2.3	1.6	1.6	1.6	1.4
Sourness	1.4	1.7	1.9	1.9	1.8	1.8	1.5	1.6	1.6
Hoppy	1.4	1.1	1.3	1.0	1.4	1.1	1.1	1.4	1.3
Fruity-esteric	1.5	1.2	1.1	1.6	1.6	1.5	2.0	1.5	1.4
Yeasty	1.1	1.2	0.9	1.2	1.6	1.3	0.8	1.1	0.9
Malty	0.4	0.7	0.8	1.1	1.5	1.1	1.1	1.1	0.7
Perfume	1.0	1.1	0.7	*	*	1.2	1.5	1.3	0.9
Caramel	-	-	-	*	*	*	*	*	*
Grainy	*	*	0.9	0.8	1.0	1.2	0.9	1.4	1.3
Sirupy	-	*	-	*	*	-	*	*	*
Worty	*	-	*	-	-	*	*	*	*
Overall impression	**3.5**	**3.9**	**3.4**	**3.4**	**3.5**	**3.8**	**3.3**	**3.6**	**3.8**

Descriptors: ascending scale 0–5 [none – very strong]; Overall impression. Descending scale 1–9 [1 – excellent; 9 – inappropriate]
– non-detected; *detected by a lower number of evaluators than the minimum for evaluation

torical varieties Opavský Kneifel and Stupický staročeský (1.4 and 1.3 points) was slightly higher than from the other varieties (0.0–1.1 points). The worty flavour was noted by some of the evaluators in beers made from malts of the Bojos, Malz and all four historical varieties.

The overall sensory impression was in a relatively narrow range from 3.3 (Chlumecký) to 3.9 (Francin) points on a descending nine-point scale, these were very good beers. Beers made from malts from the Diamant and Stupický staročeský varieties (both 3.8 points) were rated slightly worse than most other beers (3.4–3.6 points). The differences were not statistically significant at P=0.05. The result for the current varieties is in line with the recent finding from a four-year study of spring barley varieties registered in the Czech Republic stating that the sensory quality assessed by the overall impression does not differ in experimental beers from the varieties Bojos, Laudis 550, Malz, and Petrus (Mikyška et al., 2019).

Cluster analysis of the sensory profile of the beer (Figure 1) distinguished the beers made from the Malz and Petrus malt samples from the beers from other varieties. Further, all the beers from the historical varieties were separated from the beers made from Bojos, Francin and Laudis 550 malts. Of the beers from historical varieties, the smallest difference was in the profile of beers from the Diamant and Opavský Kneifel varieties.

4 Conclusion

In the 19th century, the laws of inheritance were discovered (Mendel, 1866). Knowledge of these laws was subsequently used to obtain barley varieties with better characteristics, agronomic traits (resistance to diseases and lodging, yield, etc.) but the varietal characteristics that affect the taste, colour, aroma, etc. of the final product were neglected. Research on historical varieties re-addresses this issue.

The experimental pale lager beers made from samples of four Czech historical barley varieties had a very good sensory quality, in the overall impression fully comparable to beers prepared from samples of malts of currently grown Czech barley varieties recommended for the production of beer with the PGI 'České pivo'.

This study demonstrates the continuity of the breeding of Czech barley and the preservation of the sensory characteristics of traditional Czech beer. Beers made from malts of historical varieties can be used in microbreweries or regional breweries, where they can expand the brand portfolio.

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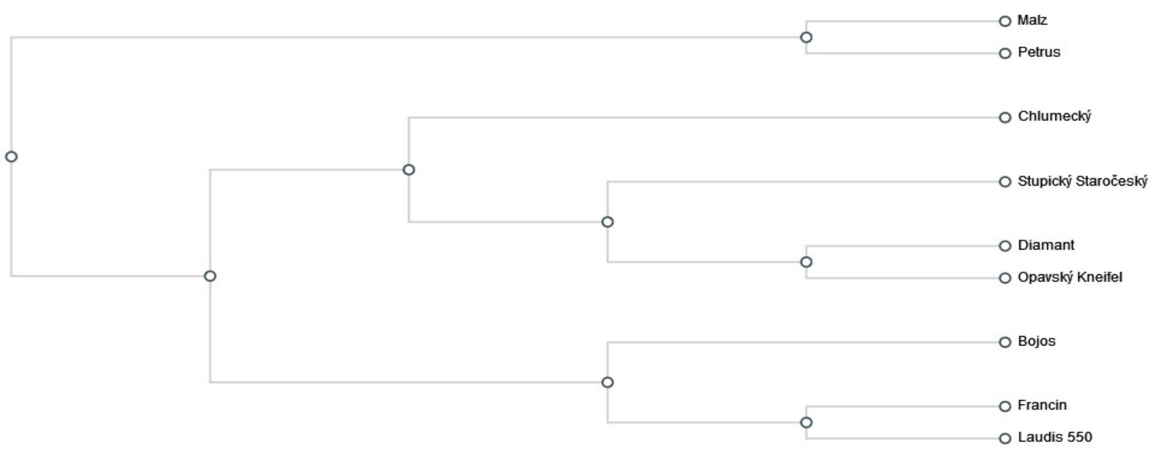
Figure 1 *Cluster analysis of sensory descriptors of experimental beers*



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