The Phenomenon of Czech Beer: a review

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Abstract

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The character and authenticity of the Czech beer, which has been accorded the protected geographical indication (PGI) České pivo by the EU, are based on specific technology and use of unique raw materials. A number of chemical and sensorial markers of the Czech beer differ from those of other lager or Pils-type beers. The majority of Czech beers contain residual (unfermented) extract. One of the most typical characteristics of Czech beers is the difference in attenuation; its long-term recorded limit value is 4.5%. Another important characteristic typical of the Czech beer is bitterness, which is mostly higher in comparison with other lagers, with the limit value at 29 EBC units. Also the colour, pH, and total polyphenol content are higher in the Czech-type beer. The limit parameters obtained by long-term monitoring were 11.8 EBC, 4.52 and 153 mg/l, respectively. Differences in amino acid and protein profiles and contents were also observed.

Keywords: Czech beer; chemical profile; sensorial profile; statistic analysis; Czech barley cultivars; Czech hop cultivars

Czechs consider the Czech beer a national beverage and part of the cultural heritage. The traditional Czech beer differs from other products in its sensorial properties that are given by specific technology and use of unique raw materials. The excellent two-rowed spring barley and the malt produced from it, along with the high-quality Saaz hops, were the basic raw materials that, together with the decoction mashing, bottom fermentation and long cold maturation produced a unique type of beer called, according to its origin in 1842 (Jalowetz 1999), Pilsner (Pils). Due to its quality it gradually spread not only all over Europe but actually worldwide. The Czech brewing industry flourished until World War I, the production in the Czech lands in 1912 reaching the record level of 11 mill. hl brewed in 650 breweries. Pilsner Urquell produced 1.3 mill. hl and in 1913 it was the largest brewery in Europe. Further dynamic technical development and concentration of the beer industry was stopped by World War II (Hönigová 2009).

Although the post-war development had a negative effect on the economic progress in the Czechoslovakia, the brewing industry paradoxically noted one positive impact – the conservation of the traditional beer production. Whereas free-market European breweries had to focus on new technologies to be able to offer competitive prices, Czechoslovak breweries created a market where the beer price was set by a government authority. The investments were low and the breweries retained the pre-war process equipment and traditional technology, including the export of breweries Budvar (Budweiser) and Plzeňský Prazdroj (Pilsner Urquell). As a consequence of the isolated Czech brewing environment, the sensory profile of the Czech beer had remained quite stable from 1938 to 1989 and the Czech beer produced in 1989 was likely to have the same character as the beer produced in central Europe in 1938. In particular, the sensory profile of the Czech beer is dominated by malt and hops, yeast or ester being acceptable. It has a medium- to full-body (mainly thanks to the content of unfermented residual extract) with slowly releasing carbon dioxide and lower alcohol content. Bitterness is medium to highest, with a moderate to light tartness, which takes longer to fade. Another feature is rich natural creamy foam (Hönigová 2009).

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Basic chemical profile of the Czech beer

During the last decade, a group of Czech researches endeavoured to specify the sensorial and analytical parameters and determine the technological factors that have the greatest influence on the characteristics of the Czech beer. The impact of their findings was, however, strongly reduced by the fact that these studies were published in local journals. The first complete study, published in 2004, compared the analytical parameters of the Czech and foreign beers of Pils type (Čejka *et al.* 2004). This study described the main Czech brands of beer using advanced analytical, sensorial and statistical methods and systematically explored the difference between Czech beers as a whole, and foreign beers of lager or Pils type. The article contains and compares the results of two studies, which were performed in 1999 and 2002. An analytical-statistical model designed to permit thorough characterisation of similarities and differences between various beers was improved and extended for the purpose of this study. Mathematical–statistical methods such as dispersion analysis, factor analysis, cluster analysis, classification trees, etc., were used for the evaluation of final results.

The 1999 study was performed on 66 beers, out of which 32 were Czech beers and 34 foreign ones. The selected beers were solely bottom-fermented, lager – or Pils-type beers from well-known breweries that were produced in large volumes and exported. This selection guaranteed beers with standardised parameters in each batch and involved the comparison of similar type beers. The study was repeated in 2002 with a smaller set of samples in order to assess any changes that could influence the first evaluation. Table 1 summarises the analytical parameters used for the beer characterisation together with references to the relevant analytical methods. It also shows if the statistical significance of a given parameter was found (Y/N); this information is based on the statistical processing described below.

The individual analytical and sensorial parameters obtained from the two beer groups (Czech and foreign) were compared using the Student's t-test. The analytical parameters which showed statistically important differences included attenuation difference, colour, pH, bitter substances, total polyphenols, acetaldehyde and pentanedione levels (Table 1). Their values were in each case higher in the Czech beers. The only trait which was higher in the group of foreign beers was the concentration of propanol.

Thorough statistical processing of the data was performed in order to find possible hidden relations between the variables as well as between the individual objects. In both 1999 and 2002 experiments, factor analysis repeatedly divided the beers into two groups (Figure 1). The group on the left contains most of the Czech beers (CZ); the group on the right contains most of the foreign ones (F). The method of cluster analysis also readily separated the brands from one another; the scatter inside the brands was usually lower than the inter-brand scatter. The results of factor analysis were confirmed by cluster analysis which showed, on one hand, the similarity between the Czech beers and, on the other, the similarity between the studied foreign beer brands.

Table 1. Comparison of analytical parameters in Czech (CZ) and foreign (F) beers from the study of Čejka *et al.* (2004)

Compared parameter	Statistical significance (Y/N)	Higher content found in	Analytical method (Reference)
Attenuation difference	Y	CZ	EBC 9.7 (2010)
Colour	Y	CZ	EBC 9.6 (2010)
pH	Y	CZ	EBC 9.35 (2010)
Bitter substances	Y	CZ	EBC 9.8 (2010)
Total polyphenols	Y	CZ	EBC 9.11 (2010)
Acetaldehyde	Y	CZ	EBC 9.39 (2010)
Pentanedione	Y	CZ	EBC 9.24.2 (2010)
Propanol	Y	F	EBC 9.39 (2010)
Diacetyl	N	–	EBC 9.24.2 (2010)
Sulphur dioxide	N	–	Dvořák *et al.* (2006)
Haze	N	–	EBC 9.29 (2010)
Dimethylsulfide	N	–	EBC 9.39 (2010)
Esters	N	–	EBC 9.39 (2010)
High and aromatic alcohols	N	–	MEBAK 9.24.2 2012)

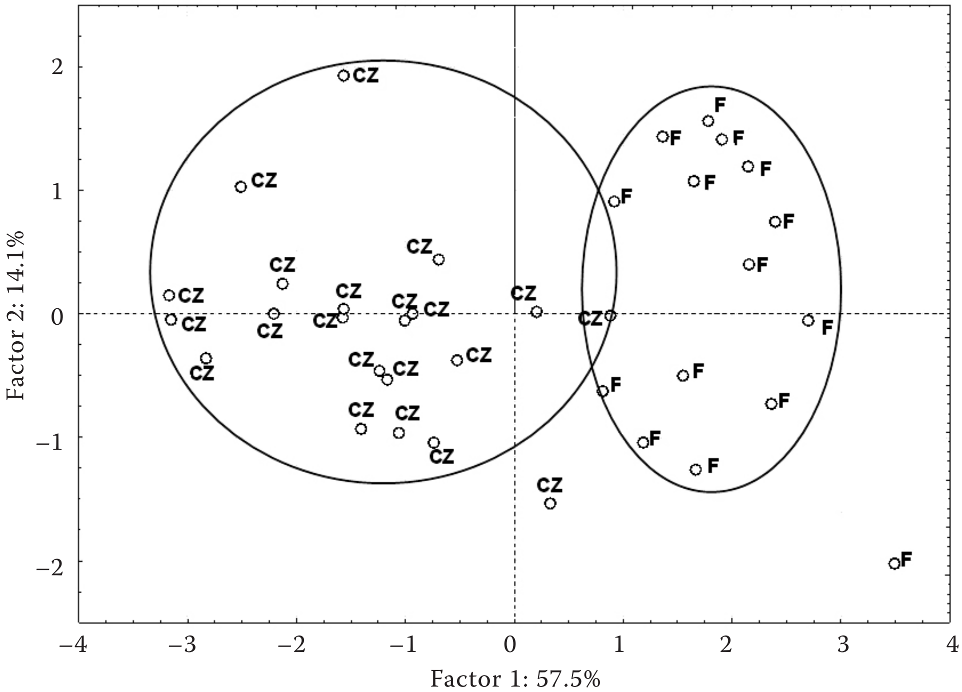

Figure 1. Factor analysis of analytical parameters of Czech (CZ) and foreign (F) beers

Discrimination analysis, which can divide the individual objects into groups according to the selected parameters, was used as another statistical method to evaluate the chemical parameters. Five parameters (attenuation difference, colour, pH, content of bitter substances, and content of polyphenols) were selected to perform the discrimination analysis of the two previously characterised beer groups. The pseudo-three-dimensional picture shows the classification based on discrimination analysis using three selected factors (out of the five). The two groups were clearly separate. Fuzzy clustering contour diagram and the "clusplot" also confirmed the separation of the two beer groups.

Sensorial profile of Czech beer

In their study, Čejka *et al.* (2004) also evaluated sensorial characteristics of the Czech beer. Table 2 summarises the sensorial parameters assessed during the sensorial analysis, and the consequent results. Although the results of sensorial analysis showed a much higher scatter than the results of chemical analysis, it was still possible to infer various hidden relations between the individual objects (beers) using statistical methods. The results of the factor analysis correlate with the analytical results; the beers were repeatedly divided into two major groups. Some of the brands could be readily distinguished from one another. It should be noted that the sensorial evaluation was done by a Czech sensorial panel and should therefore be considered partially subjective (especially as concerns the assessment of the presence of various foreign flavours and aromas).

Based on the results, it was determined that the Czech beers are more full and bitter with a longer fading of bitterness. On the other hand, foreign beers showed a higher incidence of sour and sweet flavours. Most of the foreign beers showed the presence of various flavours and aromas which are less acceptable in the Czech beers.

Defining the parameters of Czech beer

The chemical and sensorial parameters of the Czech beer characterised in the above study (Čejka *et al.* 2004) were used to define the Czech beer. The

Table 2. Comparison of sensorial parameters in Czech (CZ) and foreign (F) beers from the study of Čejka *et al.* (2004)

Sensorial parameters	Statistical significance (Y/N)	Parameter higher in
Bitterness	Y	CZ
CO_2 bite	N	–
Fullness	Y	CZ
Sweetness	Y	F
Sourness	Y	F
Off-flavours	Y	F

Sensorial analysis was performed according to Analytica EBC, Sensory analysis, EBC 13 (2010)

Table 3. Comparison of limit values of chemical parameters in Czech (CZ) and foreign (F) beers from the study of Čejka *et al.* (2004)

Chemical parameter	Limit value
Attenuation difference (%)	F < 1.0 < CZ
Colour (EBC units)	F < 9.0 < CZ
Bitter substances (EBC units)	F < 22 < CZ
pH	F < 4.45 < CZ
Total polyphenols (mg/l)	F < 135 < CZ

resultant limit values of all statistically significant parameters are summarised in Table 3. The majority of the Czech beers contain residual (unfermented) extract, whereas the majority of foreign beers are almost completely fermented. The difference in attenuation was stated to be one of the most typical characteristics of the Czech beer; its limit value is 1%. Another important characteristic typical of the Czech beer is bitterness, which is mostly higher, the limit value having been determined as 22 EBC units. Naturally, there exist special types of lager with bitterness around 35–45 EBC units produced in several countries, e.g. in Germany, but these brands are mostly specialities, not mass-produced beers. Also the colour, pH, and total polyphenol content are higher in the Czech beer. The limit parameters were assessed as 9.0 EBC, 4.45 and 135 mg/l, respectively.

Protected geographical indication České pivo

Based on a longitudinal study, the Czech beer was included in the list of foodstuffs from the Czech Republic that gained the protected geographical identification (PGI) from the European Union. The application by the Czech Republic for the registration of the name PGI České pivo (the Czech beer in Czech language) was published in the Official Journal of the European Union C 16 in line with the EC regulation no. 510/2006 on January 23, 2008 (Commission regulation 2008), and subsequently the name PGI České pivo was entered in the Register of the Protected Designations of Origin and Protected Geographical Indications. The Commission regulation no. 1014/2008 of October 16 2008 was published in the Official Journal of the European Union L 276/27 (Other Acts Commission 2008). The designation PGI České pivo is not used for all beers brewed in the territory of the Czech Republic, but only for those ones that are produced following the procedure presented in the application and fulfil the parameters given in the application.

Henceforth, the PGI term České pivo will be used in the case of direct context with the PGI while the term the Czech beer will be used when the text concerns Czech beer in general.

Barley cultivars suitable for PGI České pivo

In 2003, members of the Commission for the Evaluation of Malting Barley Varieties at the RIBM (Research Institute of Brewing and Malting, Prague, Czech Republic) described the parameters which should be met by the cultivars suitable for the production of PGI České pivo (Psota 2003). Only the cultivars Amulet and Tolar neared the parameters (extract in malt min. 81.5%; relative extract at 45°C max. 38%; Kolbach index 36–42%; diastatic power min. 220 u. WK; apparent final attenuation max. 82%; friability min. 75%, β-glucans in wort max. 250 mg/l). The values of the individual parameters were lately presented at the 9th International Barley Genetics Symposium (Kosař *et al.* 2004). During the following period, the members of the Commission for the Evaluation of Malting Barley Varieties at the RIBM chose further cultivars suitable for the production of the Czech beer, in particular the cultivars Bojos and Radegast (Psota *et al.* 2005), cv. Aksamit (Psota & Horáková 2007), and finally cv. Malz (Psota & Jurečka 2002) and cv. Calgary (Psota & Jurečka 2003).

The effect of malt used for beer brewing was briefly discussed by Čejka *et al.* (2004). A more comprehensive study was published by Psota (2008); the author tried to determine if there are any varieties suitable for the production of PGI České pivo and if there are differences between the parameters of the current and old European varieties. Therefore, he compared four sets of barley cultivars; (1) old barley cultivars (OV), (2) cultivars recommended for the production of PGI České pivo (CB), (3) European malting cultivars included in the list of the recommended cultivars of the Czech Republic (EU), and (4) cultivars included in the EBC experiment (EBC). OV differed significantly from the other cultivars tested. The sets of the EU and EBC cultivars were relatively similar; in the sum of their technological characteristics they differed from the CB set of cultivars. The set of CB cultivars differed from the other sets in a lower level of relative extract at 45°C. Together with the OV set, they differed from the EU and EBC sets in a lower level of proteolytic modification. Wort quality in the CB and EU sets was similar but it differed from the OV and EBC sets.

To sum up, current global and European requirements for the quality of brewing barley prefer cultivars with a high enzymatic activity, a high extract content, and high values of final attenuation. Barley cultivars determined for the PGI České pivo production are characterised by a lower level of proteolytic and cytological modifications and a lower level of attenuation resulting in the presence of residual extract in the final product. The fact that at least 80% of the total amount of malt grist is formed by malt made from the approved varieties guarantees a vital base for the taste (flavour) profile formation of the PGI České pivo.

Hop cultivars suitable for PGI České pivo

The characteristic bitterness of PGI České pivo is due to the wort being hopped only in the brewhouse. No processed hop products are used in the so-called cold hopping. The beer has to contain at least 30% hops cultivars suitable for the Czech beer, particularly those grown in the selected areas around Žatec, Úštěk, and Tršice. The hop is grown in loam to clay-loam soils. Permian red soils are typical of the Žatec region. The most favourable average annual temperature for hop growing is 8–10°C. The hops are quite distinctive and differ from those grown elsewhere in the world, chiefly on account of their alpha-bitter to beta-bitter acids ratio. While this ratio for commonly grown varieties is generally 2.5 : 1, that for the hops grown in this area is on average 1 : 1.5. Another feature which distinguishes them from other hops is the beta-farnesene content of 14–20% in the total essential oils. The hop cultivars cultivated in the area concerned and, in general, all hop varieties for PGI České pivo production must be approved by the supervisory authorities (Other Acts Commission 2008).

Technology used for the PGI České pivo

The specificity of PGI České pivo technology is summarised in Figure 2. The boxes highlighted in green represent the key parameters of the technology. The first parameter, spring two-row barley, was discussed in chapter 6 (Barley varieties suitable for PGI České pivo). The Pilsener type of malt (ČSN 566610:2009), which has a lower level of proteolytic and cytological modifications and a lower level of attenuation, is prepared solely from the approved barley cultivars. The longer curing phase at temperatures ranging from 80°C to 82°C is characteristic of this

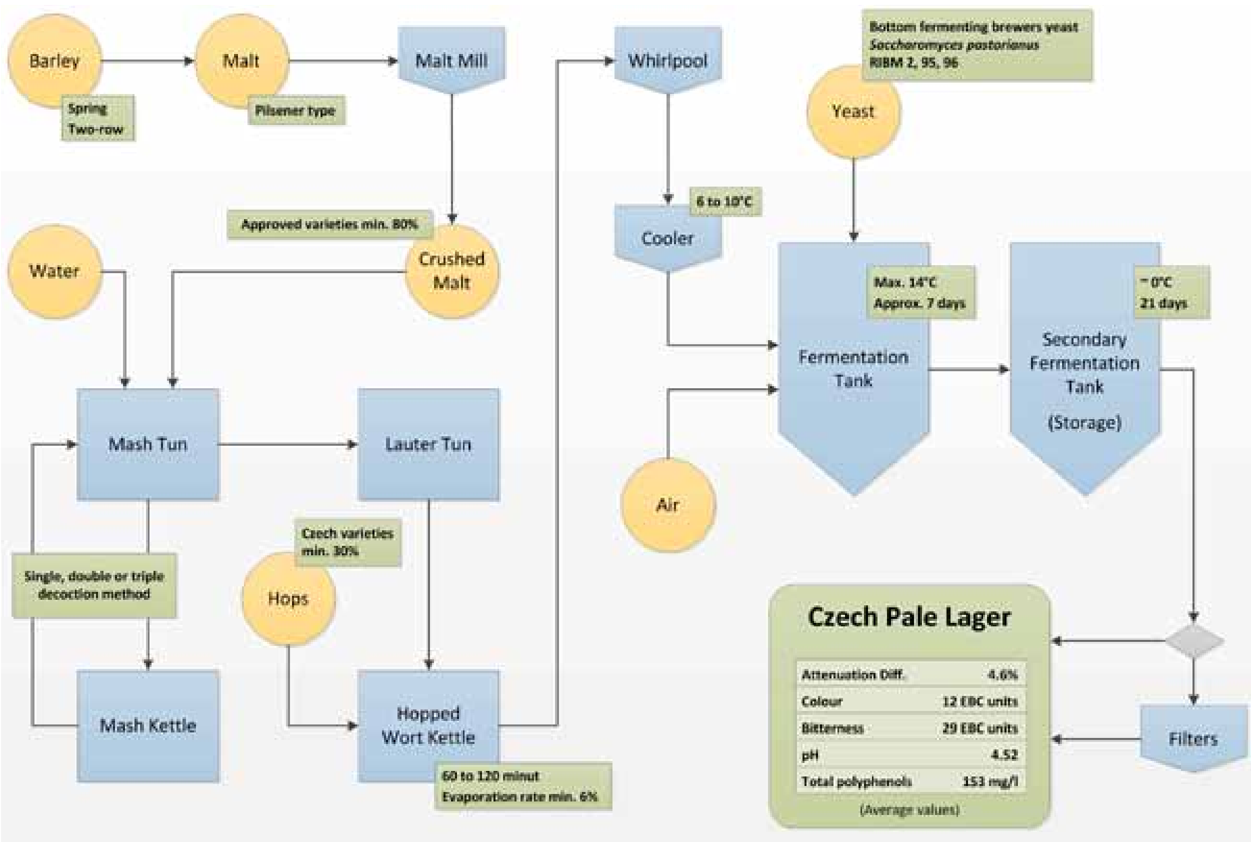

Figure 2. Scheme of Czech beer technology

malt, and causes a higher colour of the malt (Prokeš 2000). At least 80% of the total malt grist is made up of malt produced from the approved cultivars, which guarantees the taste profile of PGI České pivo. However, almost 100% of malt is usually used in Czech brewing houses for mashing. The mashing process itself employs a one-mash to three-mash decoction method; infusion mashing is not used. When the mashing process has been completed, insoluble malt particles are usually separated by lautering. During the preparation of the wort by boiling the sweet wort with the hops, which takes from 60 to 120 min (mostly 90 min), an evaporation rate of at least 6% must be achieved. Hops can be added in up to three stages. The minimum quantity of the Czech hops or products processed from them is 30% for pale lagers and at least 15% for other types of beer. Afterwards, the hopped wort is cooled down to a pitching temperature of 6–10°C and aerated. Brewer's yeast used exclusively for bottom fermenting (*Saccharomyces pastorianus*) is then added (Matoulková & Šavel 2007). The fermentation takes place at maximum temperature of 14°C and this technological process is normally separated from the secondary fermentation, i.e. two-stage fermentation is used. The secondary fermentation process is performed at temperatures close to 0°C. On completing the process of maturation by the secondary fermentation in tanks, the beer is filtered and casked, bottled, canned, or tankered. It is also possible to make unfiltered beer.

Advanced studies of chemical profile of the Czech beer

Sensorial authenticity of the Czech beer induced several authors to finding other markers of the Czech beer. Therefore, many studies dealing with the characterisation of the chemical profile of Czech beer and its comparison with other types of beer have been published. A brief overview is summarised in the following text.

Phenolic and polyphenolic compounds, flavonoids. A higher content of polyphenols in the Czech beer is undoubtedly an important health benefit. Polyphenol compounds of malt (Kaneda *et al.*1995; Boivin 2008) and hops (Lermusieau *et al.* 2001; Mikyška *et al.* 2011) affect the antioxidative activity and sensorial stability of beer. According to the literature, some 70–80% polyphenols in beer come from malt, 20–30% being hops polyphenols (De Keukeleire 1999). Polyphenol antioxidants exert significant effects on the sensorial aging of beer and contribute to the beneficial effects of beer drinking. Polyphenols can act via three mechanisms (Bamforth *et al.* 1993); as quenchers of free oxygen radicals – reactive oxygen species (ROS), as inhibitors of lipoxygenases catalysing the oxidation of fatty acids, and as chelating agents suppressing the transport of metal ions that catalyse oxidative reactions (iron, copper).

Barley is the primary source of water-soluble polyphenol antioxidants and other reducing substances. In the course of barley germination, enzyme activities bring about the release of polyphenols; the proportions of the individual fractions of water-extractable polyphenols in barley and malt are different (Dvořáková *et al.* 2008). An important tenet of the brewing industry is the monitoring of the contents and properties of the compounds that are transferred from malt into the solution during mashing. Based on the specification of the analytical parameters, the lager beer denoted the Czech beer has to contain from 130 mg/l to 230 mg/l total polyphenols. During the wort boiling, fermentation, and beer maturation, part of the wort polyphenolics are excluded from the solution and another part are removed during the colloidal stabilisation of beer by polyphenol sorbents. The content of soluble polyphenols in malt is thus a factor important for the beer quality.

Kellner *et al.* (2010) compared the contents of simple polyphenolic compounds in Czech and Bulgarian beers with other ones. Firstly, they described again a unique composition of the Czech beer, because they found that the composition of Bulgarian lager beers resembled more foreign lager beers than Czech lager beers, as evident from the colour, lower attenuation difference, content of bitter compounds, and also of total polyphenols. Moreover, they compared the content of simple polyphenols in the analysed beers. They concluded that the content of simple polyphenols in beers varies considerably and, unfortunately, they did not find any relationship with, e.g., the degree of attenuation, the content of total polyphenols or the content of anthocyanogens.

Mikyška *et al.* (2010) studied the antioxidative activity, content and composition of polyphenol compounds in malts prepared from a set of six barley varieties recommended for the production of the Czech beer and collected from four localities (experimental stations). In laboratory worts prepared by a standardised process, they determined the content of polyphenol compounds, reductive capacity by the procedure using 2,6-dichlorophenolindophenol (RC-DCPI) (Brautechnische Analysenmethoden 1987), and antiradical activity using the radical 1,1-diphenyl-2-picrylhydrazyl (ESR-DPPH) (Jurková *et al.* 2011). The results revealed varietal differences and a significant effect of soil and climatic conditions on the parameters under study. The highest values of total polyphenols, flavonoids and a relatively low content of anthocyanogens (polyphenols with the highest polymerisation index), as well as the highest values of ESR-DPPH and RC-DCPI, were found in the cv. Aksamit. The cultivars Blaník, Bojos, and Advent were comparable in terms of the content of polyphenol compounds and reducing properties. The older cv. Tolar exhibited a relatively low content of total polyphenols and a low ESR-DPPH value but a high value of RC-DCPI. The lowest content and the least farourable composition of polyphenol compounds, as well as the lowest values of ESR-DPPH and RC-DCPI, were determined in the cv. Radegast. The antiradical potential ESR-DPPH showed the strongest correlation with the content of total polyphenols, RC-DCPI correlated most closely with the content of flavonoids in the wort. The content and composition of polyphenolics in the wort were locality-dependent.

Obruca *et al.* (2009) studied phenolic compounds in beer in more detail. They analysed not only the levels of total polyphenols, but also total flavonoids and total antioxidant capacity in 7 different kinds of beer. Significantly lower phenolic and flavonoid levels were obtained in beers which do not belong to the group of the Czech beer. Further, individual phenolics were analysed using LC-ESI/MS in negative mode. The authors found 24 individual phenolics. 15 of them were identified according to their molecular weights and retention times after verification with commercially available standards. All beers contained catechin, ferrulic acid, 4-*O*-methylxanthohumol, xanthohumol, and rutin. Most of the beers contained 5,7-di-*O*-methylnaringenin, epicatechin, 5,7-*O*-methyl-8-prenylnaringenin, *p*-coumaric acid, and other unidentified polyphenols. Three other phenolic compounds, isoxanthohumol and two non-modified phenolics (MW 180 and MW 352) were not detected in foreign beers but were found only in beers produced using the traditional Czech technology. The authors concluded that some individual phenolics could be used for the verification of the beer authenticity.

Márová *et al.* (2011) investigated this issue later in more detail. They used a newly modified on-line coupled HPLC-PDA-MS method for the analysis of the characteristic phenolic compounds in several Czech lager beers, in comparison with some foreign lager beers. After optimisation of the method they identified a total of 49 compounds in the beer samples under study. Eleven individual compounds, predominantly malt phenolics (gallic acid, (–)-catechin, epicatechin, ferulic acid, chlorogenic acid, morin, rutin, quercetin, caempherol, naringenin, and luteolin) were quantified using both detection techniques. Compared with foreign beers, Czech beers contained higher levels of most of the phenolic compounds; specific distributions of the individual compounds were also observed. Experimental PDA results for the individual polyphenols were evaluated statistically by modified cluster analysis. Because of the very tight covariance of the data, a new procedure was devised for the correlation analysis. The set of beers analysed could be divided into four clusters closely related to the origin of the beer and technology used. Subgroup I contained predominantly typical Czech beers (Bernard, Gambrinus, Budweiser Budvar, and Velkopopovický Kozel). In contrast, subgroup II contained only beers produced in foreign countries by a technology different from the Czech technology. The small subgroup III contained one foreign beer (Heineken) and two Czech beers produced by a similar non-Czech technology. Subgroup IV included both Pilsner Urquell beers and two Czech non-alcoholic beers (Bernard and Staropramen). Based on MS data, no morin, luteolin, caempherol, or quercetin and very small amounts of naringenin and phenolic acids were detected in the beers of subgroups II and III. Higher values of morin were found in all samples included in subgroup I (Czech beers). Caempherol was detected only in beers of subgroup IV (Czech beers); very large amounts of naringenin, ferulic acid, and chlorogenic acid were also found in this group. The authors concluded that the flavonoids can have a potential use in the beer authenticity analysis.

The contents of prenylflavonoids (xanthohumol, desmethylxanthohumol, isoxanthohumol) were investigated in all Czech hop cultivars in 2000–2003 (Krofta 2005). The concentration of xanthohumol varied between 0.2% and 1.1% (w/w) with the highest amounts found in the new Czech high-alpha hop cultivar Agnus. The highest ratio of xanthohumol/alpha acids, greater than 10^{-1}, was determined for cv. Sladek. A positive correlation was observed for the xanthohumol and alpha acids. Fresh hops contain also a small amount of isoxanthohumol (up to 2×10^{-2}% w/w). Desmethylxanthohumol was present in Czech hop cultivars in the range of 0.50% to 0.20%

(w/w). The contents of prenylflavonoids, predominantly isoxanthohumol, in Czech beers was usually between 0.3 and 1.9 mg/l. Low isoxanthohumol levels were found if hop CO_2 extracts have been used. Prenylflavonoids were very minor constituents in draught or diet beers. The loss of isoxanthohumol in the beer production was the highest during wort cooling and trub removal.

In a study focused especially on the development of analytical methods, Česlová *et al.* (2009) presented a qualitative and quantitative analysis of hop extracts of typical Czech cultivars and Czech beer samples. Two chromatographic methods were optimised for the separation of the compounds with a lower polarity in beer and hops, and those with a higher polarity in beer. APCI-MS in both polarity modes and tandem mass spectrometry (MS/MS) were used to identify unknown prenylflavonoids. The main prenylflavonoids, xanthohumol, isoxanthohumol, and 8-prenylnaringenin together with bitter acids (α-, β- and iso-α-acids) were quantified in hop extracts and beer samples by HPLC with UV and MS detection. Four extraction techniques of hops were compared. Seven different extracts from Czech hops and six Czech beers were analysed and comparable results of both MS and UV detection were obtained. The concentration (from 0.48 mg/l to 1.79 mg/l) of isoxanthohumol in Czech beers was similar as that given in a previous work (Márová *et al.* 2011).

Significant differences between the Czech and foreign beers were found in the occurrence and average concentrations of antioxidants, phenolic compounds, and flavonoids in the study of Škeříková *et al.* (2004). For this purpose, an HPLC method was developed using a CoulArray detector with simultaneous recording of current responses of eight electrodes in series, with different potentials applied. The optimised method was used for the screening of 40 beer samples, 27 Czech and 13 foreign ones. The frequency of occurrence (%) of the studied compounds in the Czech and foreign beers was compared. The foreign beers were found to contain more frequently 4-hydroxyphenylacetic acid, vanillic acid, chlorogenic acid, vanillin, coumaric, and ferulic acid. On the other hand, protocatechuic, caffeic, and sinapic acids were determined more frequently in the Czech beers. The frequencies of other compounds were comparable. Additionally, the average concentrations of the monitored compounds were compared in both types of beer. While the concentrations of vanillic acid and vanillin were higher in the foreign beers, the concentrations of the other compounds except for 4-hydroxyphenylacetic, chlorogenic and salicylic acids and rutin, which were comparable in both beers, were found to be higher in the Czech beers.

Amino acids. Erbe and Brückner (2000) investigated the pattern and quantities of amino acid enantiomers in different types of beer and raw materials (hops, barley grains, malts) using gas chromatography (GC) on a chiral stationary phase, accompanied by high-performance liquid chromatography. Although L-amino acids were most abundant, certain D-amino acids were detected in all beers and most of the raw materials. Individual groups of beer showed very different and characteristic amino acid patterns including the ratios of enantiomers. In comparison to special beers, the common lagers, ales, and Pils beers contain lower absolute and relative amounts of D-amino acids. The total amino acid content of German lagers and Pilseners ranged from 1275 mg/l to 2260 mg/l, whereas British ales contained considerably lower amounts, i.e. 553–785 mg/l. Moderate amounts of amino acids ranging from 560 mg/l to 1080 mg/l were detected in wheat beers. The authors concluded that the quantities and patterns of amino acid enantiomers are strongly dependent on the raw materials used and, in particular, on the brewing processes employed and microorganisms involved. Based on these results, it is probable that the chemical profile of amino acids and their optical isomer forms in the typical groups of beers, such as the Czech beer, could differ. Unfortunately, such a detailed study has not yet been presented.

Some findings in this work correlate with a later publication (Kabelová *et al.* 2008) dealing with the comparison of amino acid contents in Czech end foreign beers. While the concentration of free amino acids in beers depends *inter alia* on the fermentation process (Erbe *et al.* 1999; Erbe & Brückner 2000), they contribute to the final taste properties of beers. A total of 36 beer samples were characterised, out of which 26 beers were of Czech origin and 10 were imported. Most tested beers were lagers. The contents of 16 free amino acids in 35 beers commercially available in the Czech Republic were measured using high-performance liquid chromatography with the pre-column derivatisation by AccQ Tag agent and separation on reversed phase column followed by fluorescent detection. The content of proline, which was the amino acid most commonly found, varied from 40 mg/l to 250 mg/l. The Czech beers were characterised by a significantly higher amino acid content in comparison to the imported global brands (450.41 ± 27.66 mg/l vs. 257.53 ± 46.46 mg/l).

Differences in amino acid profiles of the Czech lager beers did exist, but they were attributable mainly to the original concentration of extracts. Among 16 measured individual amino acids, the concentration of nine amino acids was significantly higher ($P < 0.01$) in the Czech beer brands than in the foreign brands studied, and these amino acids belonged to three distinctive taste groups: bitter tasting amino acids (isoleucine, leucine, lysine, phenylalanine and histidine), bitter-sweet amino acids (valine and proline), and salty-umami amino acids (glutamic and aspartic acids). Thus, the authors deduced that these differences contribute to the specific taste properties of the Czech beers and are probably influenced by a specific use of raw materials, yeast strain, and technological procedure. Moreover, beer can be successfully described not only by the basic sensorial and analytical data (Čejka *et al.* 2004), but its fine character can be described also by its amino acid content.

Polypeptides and protein composition. Obruca *et al.* (2009) tried to find other potential authenticity markers of the Czech beer. Barley and malt proteins are relatively well-documented and their analysis belongs to the commercially used tests for the barley variety authenticity. Unfortunately, beer proteome has not been studied in detail. The study determined the main protein fractions (40 kDA, lower than 8–10 kDa peptides) in lyophilised beer samples using 1D electrophoresis and microfluidic electrophoresis. Pilot 2D electrophoresis analyses of some beer samples were performed for the method optimisation. 2D gels were obtained using purified protein extracts. Repeated extraction and chromatographic procedures were used to eliminate the interference of protein Z in 2D GE. Preliminary identification of about 20 spots was done using LC-MS/MS. The study showed that the main protein fractions in most of the beers tested were protein Z, LTP1, and hordein/glutelin fragments. 2D analysis of the Czech beer differed in several spots when compared with beer made by a different technology.

Silicon. A recent study about the authenticity of the Czech beer was published in 2013 by Cejnar *et al.* (2013). The authors determined silicon in the beer samples from the Czech market and in brewing raw materials and semi-products. They found that the content of silicon in barley malt depended on the barley variety and growing region. The silicon concentration in the Czech beer ranged from 16 mg/l to 113 mg/l depending especially on two factors. Firstly, the silicon content in beer increased with increasing the original wort concentration and, secondly, during decoction mashing, which is typical of Czech beer technology, silicon from malt was leached to a much greater extent than in the case of infusion mashing.

Sustainability of the characteristic profile of the Czech beer

Since the first study characterising and defining the unique chemical and sensorial profile of the Czech beer was conducted more than ten years ago, one could raise the question whether the condition is permanent, or whether the profile could not have significantly change in the meantime. The results of our present monitoring carried out exactly according to the original study (Čejka *et al.* 2004), which are listed in Table 4, show that the Czech beer (Pils, Lagers) can still be clearly distinguished from other types of foreign beer. While the ten-year respective average values of parameters such as difference in attenuation, colour, bitter substances (bitterness), pH, and polyphenol content (total polyphenols content) of foreign beers are, respectively, 1%, 7.9 EBC units, 23.3 EBC units, 4.3 and 122 mg/l, those for the Czech beers are 4.6%, 11.8 EBC units, 29.0 EBC units, 4.5, and 154 mg/l, respectively.

This steady trend confirms that, with its analytical and sensorial properties the Czech beer remains permanently distinguishable from foreign beers.

Table 4. Long-term monitoring of basic markers of Czech lager beers and their comparison with those of foreign lager beers

	Czech beer (lagers)				Foreign beer (lagers)			
	1999	2002	2007	2012	1999	2002	2007	2012
Difference in attenuation (%)	4.8	5.3	2.7	5.7	0.2	0.1	1.8	1.5
Colour (EBC units)	12.0	10.7	12.4	12.2	7.1	7.5	8.9	8.0
Bitterness (EBC units)	30.2	26.5	28.9	30.2	23.3	23.3	26.0	20.6
pH	4.51	4.56	4.48	–	4.33	4.27	4.40	–
Total polyphenols (mg/l)	153	149	133	179	122	116	104	146

CONCLUSION

Long-term research has proven that the phenomenon of the Czech beer can be largely attributed to its characteristic chemical profile. A number of studies have repeatedly confirmed that the Czech beer differs from other light lagers in its residual extract (in other words attenuation difference), higher bitterness, colour, higher content of polyphenolic substances, and higher pH. Moreover, it was determined that Czech beer clearly contains a different spectrum of polyphenols, which increase its antioxidative properties. It also possesses characteristic volatile compounds and amino acid profile that correlate with its sensorial properties. Pilot proteomic studies have indicated that the protein composition of the Czech beer also differs from that of foreign beers. Other studies are currently underway to characterise further markers of the Czech beer. In terms of the production technology, these typical chemical properties, which impart to the Czech beer its unique sensorial character, are given above all by the selection of the raw materials and the decoction mashing. Higher bitterness with its characteristic longer fading is due to brew-house hopping, cold hopping being unacceptable for the Czech beer. All these factors, which underlie the authenticity of Czech beer, are summarised in Figure 2.

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