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Comparative chemical analysis of traditional Czech beers, with and without the protected geographical indication mark "Czech Beer"

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Abstract

The present work conducted a comparative chemical analysis of traditional Czech beers with degrees Plato (°P) ranging from 10° to 12°. As some breweries in the Czech Republic may use the protected geographical indication mark (PGI) "Czech Beer" (provided that the production conditions are met), differences in the chemical composition of beers, with and without the PGI, may arise. Significant differences were observed in the chemical composition of Czech beers with different °P values. Generally, beers with 12°P exhibited significantly higher concentrations in all examined parameters, including total protein content, total carbohydrate content, total phenolic content, concentration of individual phenolic compounds, ABTS and DPPH radical scavenging capacity, organic acid concentration, and concentrations of selected elements compared to beers with 10°P. The results also indicated that beers, with and without the PGI, particularly the 12°P beers, did not differ in terms of chemical composition. This suggests that the production of these beers in the Czech Republic adheres to traditional brewing practices, using quality malt and typical Czech hop varieties.

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Introduction

Beer is one of the most popular alcoholic beverages, with production and consumption spread worldwide. In 2018, beer production in the European Union exceeded 400 Mhl, with the Czech Republic accounting for 21 Mhl, approximately 5% of European production (The Brewers of Europe, 2019). Moreover, beer consumption per capita in the Czech Republic is the highest in the EU, averaging around 130 L per year (Grosová *et al*., 2017).

Beer, as the final product of the brewing process, is a complex mixture of water and hundreds of other substances that contribute to its character. From a nutritional perspective, beer contains water, proteins, B vitamins, certain minerals, phenolic compounds, carbohydrates, and dietary fibre (Bamforth, 2002). Moderate beer consumption may provide various health benefits, such as preventing human diseases associated with oxidative stress, reducing cardiovascular disease, promoting an anti-osteoporosis effect, stimulating the immune system, and lowering the risk of dementia (Kaplan *et al*., 2000; Kondo, 2008; Sohrabvandi *et al*., 2012).

The Czech Republic has a long-standing tradition of beer brewing. Czech beer classification is based on the concentration of dissolved solids in the brewery wort, expressed in degrees Plato (°P), and distinguishes five basic groups of beers with an alcohol content above 1.2% (v/v) (Czech Republic, 2018). The most popular beers among consumers in the Czech Republic are traditional bottom-fermented beers within the 7 - 10°P or 11 - 12°P range.

Beers with 7 - 10°P have a pale colour, medium bitterness, and relatively delicate taste. Beers with 11 - 12°P exhibit clear pale gold to darker orange-gold colours, a clean aroma with a rich and complex malt presence, and a well-rounded taste with complex malt flavours complemented by spicy tones from typical Czech hop varieties. These beers are famously known as "Pilsner beers" because the recipe originated more than 100 years ago in the city of Plzeň (Pilsen).

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Czech beer is unique worldwide due to its traditional brewing process, and the use of specific raw materials. In 2008, the European Union granted the protected geographical indication mark (PGI) for Czech beer (EC Regulation No. 1014/2008). However, the PGI "Czech Beer" is not used for labelling all beers produced in the Czech Republic, but only for those that have applied for the PGI, and met the required conditions (Olšovská *et al*., 2014). One of the criteria for achieving the PGI is the use of specific raw materials. The selection of barley malt, hops, water, and yeasts is crucial for brewing a beer labelled with the PGI "Czech Beer." The Pilsner-type malt, prepared from spring two-row barley cultivars, is used for producing PGI-labelled beers. This malt undergoes an extended curing phase during kilning at temperatures ranging from 80 to 82°C, resulting in a higher malt colour. The Pilsner-type malt exhibits lower levels of proteolytic and cytological modifications, and attenuation. Recommended Czech hop varieties for beers labelled with the PGI "Czech Beer" include 'Saaz', 'Sládek', and 'Premiant'. Czech hops differ from those grown in other parts of the world due to their ratio of α- to β-bitter acids. While the commonly grown varieties generally have a ratio of 2.5:1, the average ratio for hops grown in the Czech Republic is 1:1.5 (Olšovská *et al*., 2014).

Another distinguishing feature of Czech hops is the β-farnesene content, which ranges from 14 to 20% of the total essential oils. This unique composition of Czech hops imparts a distinct sensory character to Czech beers. Water and yeast are the other raw materials used in the brewing process. Water can be sourced locally, and should meet the specified hardness requirements. Only bottom-fermented yeasts are allowed for fermentation (Matoulková and Šavel, 2007). In addition to specific raw materials, the application of certain brewing processes is essential. The traditional brewing process employed is specified as one to three mashing processes by decoction (the infusion mashing method is not permitted), followed by fermentation at a maximum temperature of 14°C in two stages.

Due to the exceptional nature of Czech beer, many researchers have studied its properties in comparison to beers from different origins (Čejka *et al*., 2004; Kellner *et al*., 2010; Márová *et al*., 2011). However, no comprehensive study comparing Czech beers, with and without the PGI designation, has been published recently. Therefore, the present work aimed to characterise beers with 7 - 12°P produced in the Czech Republic, both with and without the PGI designation, and compare them to identify any significant differences in their chemical composition.

Materials and methods

Samples

A total of 32 samples of Czech beers were analysed. Beer samples were purchased from the local market. From the total number of samples, 18 samples were beers with 10°P (six with the PGI “Czech Beer”), and 14 samples were beers with 12°P (eight with the PGI “Czech Beer”). All samples were stored at 4°C until further analyses. All samples were degassed by sonication (Ultrasonic Compact Cleaner, Notus - Powersonic, Slovakia), and filtered through a membrane filter with 0.45 μm porosity (Labicom, Czech Republic) before analysis.

Chemicals and reagents

The following chemicals and reagents were used in the present work. HPLC-grade acetonitrile was purchased from Sigma-Aldrich (St. Louis, USA). HPLC-grade formic acid was purchased from Lachner (Neratovice, Czech Republic). Folin-Ciocalteu’s phenolic reagent, α-D-glucose, anthrone, Trolox, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, USA) as high purity chemicals. Sodium hydroxide, anhydrous sodium carbonate, potassium sodium tartrate tetrahydrate, and copper sulphate pentahydrate were purchased from Lachner (Neratovice, Czech Republic) with purity ≥ 99%. Sulphuric acid (96%, high purity) was purchased from Penta (Praha, Czech Republic). Albumin Bovine Fraction V standard was purchased from Serva (Heidelberg, Germany). Standards of organic acids (acetic, citric, malic, lactic, and succinic acids) were purchased from Fluka Analytical (St. Gallen, Switzerland). Phenolic compounds (rutin hydrate, gallic acid, catechin hydrate, *trans*-ferulic acid, vanillic acid, and *p*-coumaric acid; purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, USA). Standard solutions of elements (potassium, calcium, magnesium, copper, iron, manganese, phosphorus, and silicon; 1000 ± 2 mg/L) were purchased from Analytika (Praha, Czech Republic).

Instrumentation

A Unicam Helios Gamma spectrophotometer was used for the determination of total protein content (PC), total phenolic content (TPC), total carbohydrate content (TCC), and total antioxidant activity. An ion chromatograph (Metrohm 850 professional ion chromatography (IC), Metrohm, Switzerland) with a conductivity detector was used for the determination of organic acids. A liquid chromatograph (Agilent Infinity 1260, Agilent Technologies, USA) equipped with a diode array detector (DAD) was used for the determination of individual phenolic compounds. An optical emission spectrometer with inductively coupled argon plasma (Ultima 2, Horiba Scientific, France) was used for the determination of elements.

Procedures

The PC was determined according Lowry *et al*. (1951), using bovine serum albumin as the standard. The TPC was determined using Folin-Ciocalteu's method described by Singleton *et al*. (1999), and expressed as gallic acid equivalent. The TCC was determined according to Yadav *et al*. (2018), and the results were expressed as glucose equivalent. The total antioxidant activity was determined using the ABTS and DPPH methods according to Szwajgier (2009). The total antioxidant capacity was expressed as a Trolox equivalent. The contents of acetic, citric, malic, and lactic acids were determined using IC according to Diviš *et al*. (2018). The contents of rutin, catechin, gallic acid, ferulic acid, vanillic acid, and coumaric acid were determined by high-pressure liquid chromatography (HPLC). The separation was achieved using a Kinetex EVO-C18, 2.7 µm, 4.6 × 150 mm (Phenomenex, USA) column. A mixture of 0.1% formic acid and acetonitrile (90:10%, v/v) was used as a mobile phase. The phenolic compounds were detected with a DAD. Monitored wavelengths were 280, 290, and 350 nm. Concentrations of Cu, Fe, Mn, P, Ca, K, and Mg were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Ultima 2, Horiba Scientific, France) according to Diviš *et al*. (2015). Si was also analysed by ICP-OES at 251.611 nm.

Quality control

All calibration curves used for quantification of selected analytes had a determination coefficient R^2 > 0.99. In the case of IC or HPLC, qualitative analysis was based on a comparison of the retention time of the analyte in the real sample with the retention time of the pure compound (standard). Moreover, in the case of HPLC, spectra of the selected analyte and standard from the DAD detector were compared to exclude possible co-elution of the unknown compound and analyte. The trueness of the analytical methods employed in the present work was evaluated using the recovery for a beer sample spiked with a known amount of analytes. The acceptable recovery limit of 89 - 105% was achieved for all analytes. During the analysis of real samples with the selected analytical method, an analysis of a control beer sample spiked with a known amount of analytes was performed. All samples were analysed in duplicate, and each analysis was performed in triplicate.

Statistical analysis

All experimental data were statistically processed using the software XLstat (Addinsoft, USA). Based on the Grubbs test, five samples showing extreme values in the results were excluded. The data obtained were treated using Analysis of Variance (ANOVA) and Tukey's comparative test to find potential statistically significant differences between the tested groups of beer samples. Hypothesis testing was set with a significance level of 0.05. Principal Component Analysis (PCA) based on Pearson's correlation was used for multivariate characterisation and visualisation of observations.

Results and discussion

Total protein content

The PC ranged from 3.58 to 5.82 g/L in the 10°P beers, with an average content of 4.64 g/L (Table 1). The PC in 12°P beers varied from 4.21 to 6.52 g/L, with an average content of 5.38 g/L (Table 1). Using One-way ANOVA analysis, a significant difference (p = 0.0011) in the PC among the beer samples between 10°P and 12°P beers was observed. The difference in the PC between 10°P beers, with and without the PGI, was also significant (p = 0.0159), while among the 12°P beers, no significant difference (p > 0.05) between beers, with and without the PGI, was observed. The primary source of proteins and peptides in beer is malt; however, the PC in beer depends not only on the raw materials but also on the technology used. PGI Czech beer is produced exclusively by the decoction mashing method (EC Regulation No. 1014/2008), which significantly

Table 1. Mean values and standard deviations of total protein content (g/L), total carbohydrate content (g/L), total phenolic content (mg/L), and organic acids concentrations (mg/L) of Czech beer samples.

Beer type	PC	TCC	TPC	Citric acid	Malic acid	Lactic acid	Acetic acid
12°P without PGI	5.24 ± 0.46[a]	33.4 ± 5.6[a]	562 ± 110[a]	121 ± 31[a]	45.9 ± 9.9[a]	42.7 ± 17.4[a]	35.7 ± 17.0[a]
12°P with PGI	5.52 ± 0.79[a]	33.1 ± 3.7[a]	628 ± 121[a]	164 ± 87[a]	38.8 ± 11.8[a]	49.6 ± 18.8[a]	53.9 ± 20.7[a]
12°P average	5.38 ± 0.64[a]	33.3 ± 4.5[a]	595 ± 116[a]	143 ± 66[a]	42.3 ± 11.0[a]	46.2 ± 17.7[a]	44.8 ± 20.4[a]
10°P without PGI	4.84 ± 0.16[a]	28.4 ± 5.5[a]	440 ± 38[a]	201 ± 48[a]	63.9 ± 35.5[a]	108 ± 49.9[a]	86.5 ± 24.2[a]
10°P with PGI	4.35 ± 0.49[b]	27.4 ± 1.0[a]	495 ± 15[a]	180 ± 74[a]	63.8 ± 35.3[a]	110 ± 91.7[a]	81.1 ± 36.9[a]
10°P average	4.64 ± 0.40[b]	28.0 ± 4.2[b]	462 ± 99[b]	192 ± 58[a]	63.8 ± 34.2[a]	109 ± 66.5[a]	84.3 ± 28.8[b]

Mean values with different lowercase superscripts are significantly different at $p < 0.05$.

affects the difference in the PC among 10°P beers. Beer contains about 3 - 5 g/L of proteins, such as albumins (*e.g.*, LTP proteins), globulins (α, β, γ, and δ-globulins), gluten, and various glycoproteins, all of which contribute to the total PC value. Olšovská *et al.* (2019) pointed out that, in general, the nutritional value of beer increases in 12°P beers due to higher original gravity and the amount of raw materials used for beer brewing. The results obtained in the present work agreed with those of Olšovská *et al*. (2019), who reported the PC of between 2.2 and 4.5 g/L in 10°P Czech beers, and between 3.3 and 6.1 g/L in 12°P Czech beers. A similar PC was observed by Pai *et al*. (2015) in Indian beers (2.04 - 5.41 g/L).

Total carbohydrate content

The TCC in 10°P beers ranged from 17.3 to 38.4 g/L (Table 1), with an average of 28.0 g/L. TCC in 12°P beers ranged from 27.3 to 40.4 g/L (Table 1), with an average of 33.3 g/L. The 12°P beers had significantly higher TCC ($p = 0.0047$) than the 10°P beers, while no significant difference in the TCC was observed between 10°P or 12°P beers, with and without PGI ($p > 0.05$). Carbohydrates are extracted into beer from the malt during the mashing process, and serve as the primary energy source for yeasts during alcoholic fermentation. Residual carbohydrates after fermentation, along with ethanol, contribute the most to the total caloric value of beer, and their content may vary depending on the yeast's ability to use sugars. The attenuation mainly depends on the yeast strain used, and is typically higher for 10°P beers than for 12°P beers (Černohorský *et al*., 1986). The results of the TCC analysis were comparable to those published by Olšovská *et al.* (2019), which reported the TCC in 10°P beers to be 12.3 - 38.6 g/L, and the TCC in 12°P beers to be 25.6 - 45.2 g/L.

Total phenolic content

The TPC of the beer samples varied significantly. The TPC in the 10°P beer samples ranged from 355 to 791 mg/L, with an average of 462 mg/L (Table 1). The TPC in the 12°P beer samples ranged from 476 to 830 mg/L, with an average of 595 mg/L (Table 1). One-way ANOVA analysis confirmed that 12°P beers had a significantly higher TPC ($p = 0.0034$) than 10°P beers. However, no significant difference appeared in the TPC ($p > 0.05$) between 10°P or 12°P beers, with and without the PGI designation. The difference in the TPC between 10°P and 12°P beers can be explained by the amount of raw materials used, particularly hops and barley malt, which are the main sources of compounds contributing to the TPC in beer (Kellner *et al*., 2010). Previous studies on Czech beers by other authors reported TPC ranging from 152 to 180 mg/L (Kellner *et al.*, 2010; Márová *et al*., 2011; Olšovská *et al*., 2019), which is generally lower than the values found in the present work. However, the TPC in Serbian beers (Mitić *et al*., 2013) was comparable to the TPC found in the present work (TPC: 328 - 545 mg/L). Habschied *et al.* (2020), who analysed German, Croatian, and Czech beers, found TPC in the range of 464 - 579 mg/L, which also corroborated the findings of the present work.

Individual phenolic compound content

The most abundant phenolic compound among the samples investigated was gallic acid. The concentration of gallic acid in 10°P beers ranged from 1.32 to 5.55 mg/L (Table 2), with an average concentration of 3.26 mg/L. The concentration of gallic acid in 12°P beers ranged from 2.81 to 5.79 mg/L (Table 2), with an average concentration of 4.37 mg/L. Statistical analysis of the data revealed significant differences between 12°P and 10°P beers, in terms of gallic acid concentration ($p = 0.0195$), vanillic acid concentration ($p = 0.0003$), ferulic acid concentration ($p < 0.0001$), and rutin concentration ($p < 0.0001$). No significant statistical difference was observed between beers, with and without PGI, regarding the content of individual phenolic compounds, except for ferulic acid in 10°P beers ($p = 0.0207$) and coumaric acid in 12°P beers ($p = 0.0097$). The profile of phenolic compounds in beer is influenced by the composition of raw materials and the brewing technology employed. These phenolic compounds contribute to the beer's antioxidant capacity and stability (Mikyška *et al*., 2002; Kellner *et al*., 2010), while also creating a unique sensory profile. In Czech 12°P beers, Márová *et al.* (2011) determined that ferulic acid was the most abundant phenolic compound, with an average concentration of 3.96 mg/L, whereas rutin was the least abundant, with an average concentration of 0.54 mg/L. Kellner *et al*. (2010) also found ferulic acid to be one of the most abundant phenolic compounds in Czech 12°P beers, with an average concentration of 3.75 mg/L, while gallic acid was the least abundant, with an average concentration of 0.104 mg/L. In Czech 10°P beers, Kellner *et al*. (2010) determined that ferulic acid was

Table 2. Mean values and standard deviations of phenolic compound concentration (mg/L) and antioxidant activity (mmol TE/L) of Czech beer samples.

Beer type	GA	VA	CA	FA	Rutin	Catechin	ABTS	DPPH
12°P without PGI	4.31 ± 1.2[a]	1.19 ± 0.41[a]	0.93 ± 0.42[a]	3.12 ± 0.55[a]	2.40 ± 0.45[a]	1.22 ± 1.4[a]	1.66 ± 0.26[a]	0.63 ± 0.14[a]
12°P with PGI	4.42 ± 0.40[a]	1.23 ± 0.32[a]	0.44 ± 0.22[b]	3.61 ± 1.03[a]	2.62 ± 0.82[a]	2.31 ± 1.3[b]	1.69 ± 0.21[a]	0.77 ± 0.23[a]
12°P average	4.37 ± 0.85[a]	1.21 ± 0.41[a]	0.61 ± 0.41[a]	3.40 ± 0.82[a]	2.53 ± 0.63[a]	1.73 ± 1.4[a]	1.67 ± 0.22[a]	0.71 ± 0.20[a]
10°P without PGI	3.07 ± 1.3[a]	0.73 ± 0.50[a]	0.41 ± 0.21[b]	2.32 ± 0.31[a]	1.72 ± 0.32[a]	1.62 ± 0.82[a]	1.30 ± 0.11[a]	0.32 ± 0.02[a]
10°P with PGI	3.56 ± 1.4[a]	0.45 ± 0.13[a]	0.52 ± 0.20[a]	1.63 ± 0.72[b]	1.23 ± 0.61[b]	0.92 ± 0.41[b]	1.14 ± 0.09[a]	0.35 ± 0.09[a]
10°P average	3.26 ± 1.3[b]	0.59 ± 0.41[b]	0.43 ± 0.23[a]	2.11 ± 0.64[b]	1.51 ±0.52[b]	1.33 ± 0.82[a]	1.24 ±0.11[b]	0.33 ± 0.05[b]

Mean values with different lowercase superscripts are significantly different at $p < 0.05$. GA: gallic acid, VA: vanillic acid, CA: coumaric acid, and

the third most abundant phenolic compound, with an average concentration of 2.75 mg/L, and gallic acid was the least abundant, with an average concentration of 0.106 mg/L. On the other hand, Habschied *et al*. (2020) reported the gallic acid contents in Czech and German 12°P beers to be in the range of 42 - 51 mg/L. Comparable results were published by Floridi *et al.* (2003) concerning samples of Italian lager beers, where ferulic acid was the most abundant phenolic compound, with an average concentration of 2.41 mg/L, while gallic acid was the least abundant, with a concentration of 0.593 mg/L.

ABTS and DPPH radical scavenging activity

The ABTS radical scavenging activity of 10°P beers ranged from 1.02 to 1.41 mmol of Trolox equivalent per litre (TE/L), while the DPPH radical scavenging activity varied from 0.24 to 0.47 mmol TE/L (Table 2). The ABTS radical cation scavenging activity of 12°P beers ranged from 1.40 to 2.10 mmol TE/L, while the DPPH radical scavenging activity varied from 0.52 to 1.10 mmol TE/L (Table 2). Significantly higher ABTS radical cation scavenging activity (p = 0.0022) and DPPH radical scavenging activity (p < 0.0001) were observed in 12°P beers than in 10°P beers. However, no significant difference was observed in the 12°P or 10°P beer samples, with and without PGI (p > 0.05). The antioxidant activity of beer is a parameter that describes beer freshness, stability, or ageing, and can be influenced by various factors. Pearson's correlation analysis showed a positive correlation between DPPH radical scavenging activity and PC (r = 0.6443), concentrations of rutin (r = 0.4074), ferulic acid (r = 0.4600), potassium (r = 0.5838), and magnesium (r = 0.7370). These results agreed with those published by Ditrych *et al*. (2016), who determined the DPPH radical cation scavenging activity in Polish lager beers to be in the range of 0.65 - 1.3 mmol TE/L.

Organic acid content

Organic acids are secondary metabolic products excreted by yeasts during the alcoholic fermentation of beer. They are important for the stability, pH, and taste of the beer, and they serve as biomarkers of the fermentation process, especially lactic and pyruvic acids. The most abundant organic acid in Czech beers was citric acid. In 10°P beers, the concentration of citric acid varied from 65.1 to 249 mg/L, with an average concentration of 192 mg/L (Table 1). In 12°P beers, the concentration of citric acid ranged from 63.1 to 340 mg/L, with an average concentration of 143 mg/L (Table 1). On the other hand, the least abundant organic acid in Czech beers was malic acid. In 10°P beers, the concentration of malic acid ranged from 1.92 to 95.2 mg/L, with an average concentration of 63.8 mg/L (Table 1). In 12°P beers, the concentration of malic acid ranged between 15.3 and 55.0 mg/L, with an average concentration of 42.3 mg/L (Table 1). A significant difference (p = 0.0005) was observed in the concentration of acetic acid between 10°P and 12°P beers. Acetic acid is produced rapidly during the early stages of beer fermentation, but is also metabolised later (Whiting, 1976). During the lag phase and early stages of fermentation, when aerobic respiration occurs, and cells are exposed to an excess of glucose, acetate is formed *via* the cytosolic pyruvate dehydrogenase (PDH) bypass (Remize *et al*., 2000). During the stationary phase and in substrate deficiency, acetic acid is transformed into acetyl-CoA, which is the precursor of a wide range of bioproducts (Liu *et al*., 2017). The concentration of other organic acids showed no significant difference between 10°P and 12°P beers, and the same result was observed between beers with and without PGI (p > 0.05). These were consistent with those published by Klopper *et al*. (1986), who determined organic acids in 12°P beers from the Netherlands and Denmark. In these beers, citric acid was the most abundant organic acid (148 mg/L), while malic acid was the least abundant (63 mg/L). Li and Liu (2018) also found that citric acid was the most abundant organic acid in the beers studied, with a concentration of 157.06 mg/L, while malic acid was the least abundant, with a concentration of 81.25 mg/L. The concentration of individual organic acids depends on various factors such as the initial concentration of organic acids in the wort, the yeast strain used, and the fermentation process and conditions (Whiting, 1976; Klopper *et al*., 1986; Li and Liu, 2018). The large variability in the results reported by different authors can be attributed mainly to the use of different raw materials, yeast strains, and fermentation conditions.

Elemental analysis

The most abundant element in 10°P beers was phosphorus, while the most abundant element in 12°P beers was potassium. The concentration of phosphorus in 10°P beers varied from 268 to 471 mg/L (Table 3), with an average concentration of 375

Table 3. Elemental composition (mg/L ± standard deviation) of Czech beer samples.

Beer type	Cu	Fe	Mn	P	Si	Ca	K	Mg
12°P without PGI	0.18 ± 0.15^{a}	0.18 ± 0.05^{a}	0.14 ± 0.07^{a}	423 ± 71^{a}	64.7 ± 7.6^{a}	50.5 ± 23^{a}	488 ± 55^{a}	109 ± 16^{a}
12°P with PGI	0.12 ± 0.03^{a}	0.11 ± 0.05^{a}	0.13 ± 0.03^{a}	437 ± 33^{a}	55.2 ± 14^{a}	62.9 ± 32^{a}	490 ± 74^{a}	129 ± 27^{a}
12°P average	0.15 ± 0.11^{a}	0.15 ± 0.06^{a}	0.14 ± 0.05^{a}	430 ± 54^{a}	59.9 ± 12^{a}	56.7 ± 28^{a}	489 ± 62^{a}	119 ± 24^{a}
10°P without PGI	0.09 ± 0.05^{a}	0.10 ± 0.09^{a}	0.08 ± 0.04^{a}	372 ± 63^{a}	59 ± 17^{a}	24.8 ± 11^{a}	373 ± 73^{a}	66.5 ± 13^{a}
10°P with PGI	0.06 ± 0.06^{a}	0.05 ± 0.03^{a}	0.09 ± 0.03^{a}	380 ± 55^{a}	34.4 ± 7.3^{b}	36.2 ± 24^{a}	392 ± 45^{a}	78.3 ± 13^{a}
10°P average	0.08 ± 0.06^{b}	0.08 ± 0.08^{b}	0.08 ± 0.04^{b}	375 ± 58^{b}	49.2 ± 18^{a}	29.3 ± 17^{b}	381 ± 63^{b}	71.3 ± 14^{b}

Mean values with different lowercase superscripts are significantly different at $p < 0.05$.

mg/L. The concentration of potassium in 12°P beers ranged from 387 to 597 mg/L (Table 3), with an average concentration of 430 mg/L. Significant differences were observed between 12°P and 10°P beers in the concentration of copper ($p = 0.0313$), iron ($p = 0.0241$), manganese ($p = 0.0019$), phosphorus ($p = 0.0181$), calcium ($p = 0.0041$), potassium ($p = 0.0001$), and magnesium ($p < 0.0001$). Among Czech 10°P beers, a significantly higher concentration of silicon ($p = 0.0054$) was observed in beer samples without PGI, while in 12°P beers, no significant difference in the concentrations of these elements was observed. The elemental content in the wort significantly influences the brewing and fermentation process (Alcázar, 2001). The main sources of minerals in beer are barley malt, water, hops, and yeast (Ibanez *et al*., 2008; Punčochářová *et al.*, 2019). Different post-fermentation processing technologies, especially kieselguhr filtration, can also contribute to the presence of elements in beer (Wietstock *et al*., 2018; Slabý *et al*., 2018). Although the elemental composition of Czech beers has not been extensively investigated, other authors have published comparable concentrations of elements in different types of beers. Alcázar (2001) found phosphorus to be the most abundant element in beer (average concentration of 215.83 mg/L) along with potassium (average concentration of 405.7 mg/L), while zinc and barium were the least abundant elements (average concentration of less than 1 mg/L). Asfaw and Wibetoe (2005) investigated the copper, manganese, and iron contents in beer using ICP-AES. The copper content in beer samples ranged from 0.007 to 0.049 mg/L, manganese content ranged from 0.032 to 0.142 mg/L, and iron content ranged from 0.035 to 0.175 mg/L. Wietstock *et al*. (2018) identified copper as the most abundant element in beer (0.038 mg/L), while iron was the least abundant element (0.009 mg/L). Sakellari *et al.* (2017) examined trace elements in Greek beer, and determined the concentrations of copper (0.029 mg/L), iron (0.179 mg/L), and manganese (0.157 mg/L).

Processing of measured data by multivariate analysis

PCA was performed as an extension to univariate analysis to multivariately characterise the beer samples (Figure 1). The original data were reduced into three principal components with eigenvalues > 1. The first two principal components, F1 and F2, together accounted for 73.04% of the variability in the original dataset. Component F1 showed a strong positive correlation with DPPH radical scavenging activity and the concentrations of vanillic acid, ferulic acid, rutin, potassium, and sodium. Other positive correlations were also observed. F1 exhibited a moderate positive correlation with PC and the concentration of silicon, and a weak positive correlation with coumaric acid concentration. Conversely, F1 displayed a strong negative correlation with acetic acid concentration. Component F2 exhibited a moderate positive correlation with the concentrations of ferulic acid, rutin, and silicon. It also displayed a weak positive correlation with acetic and vanillic acid concentrations. However, F2 showed a weak negative correlation with DPPH radical scavenging activity, PC, coumaric acid concentration, and the concentrations of potassium and magnesium.

The graphical characterisation of the Czech beer samples was obtained by plotting the observations in the 2D-factor plane of the principal components F1 and F2. The planar projection (Figure 2) shows that the Czech beer samples were separated into two main clusters based on their °P. The 10°P beers were projected in the left hemisphere of the 2D planar projection, while the 12°P beers were projected in the right hemisphere. This projection confirmed the results of the univariate analysis, which showed significant differences in the concentrations of certain compounds between the 10°P and 12°P beers. PCA could not effectively separate the samples with the PGI from other samples. However, some clustering was observed among the10°P beers. This could have been due to the use of more diverse technologies, such as high gravity brewing, and different raw materials in the production of 10°P beer without PGI (*e.g*., adjuncts or hop isoextracts). On the other hand, the technology for the production of 12°P beer is relatively uniform regardless of PGI status. Another reason it was not possible to effectively separate beers, with and without PGI, could be that the PGI "Czech Beer" is based on Czech beer brewing traditions that originated in the nineteenth century. While not every Czech brewery uses this PGI, most breweries follow the same traditional beer brewing procedures that are deeply rooted in the entire industry. Additionally, Czech customers are very conservative, and have specific sensory parameters for Czech beer, which are developed based on the specific beer brewing process and raw materials used in beer production.

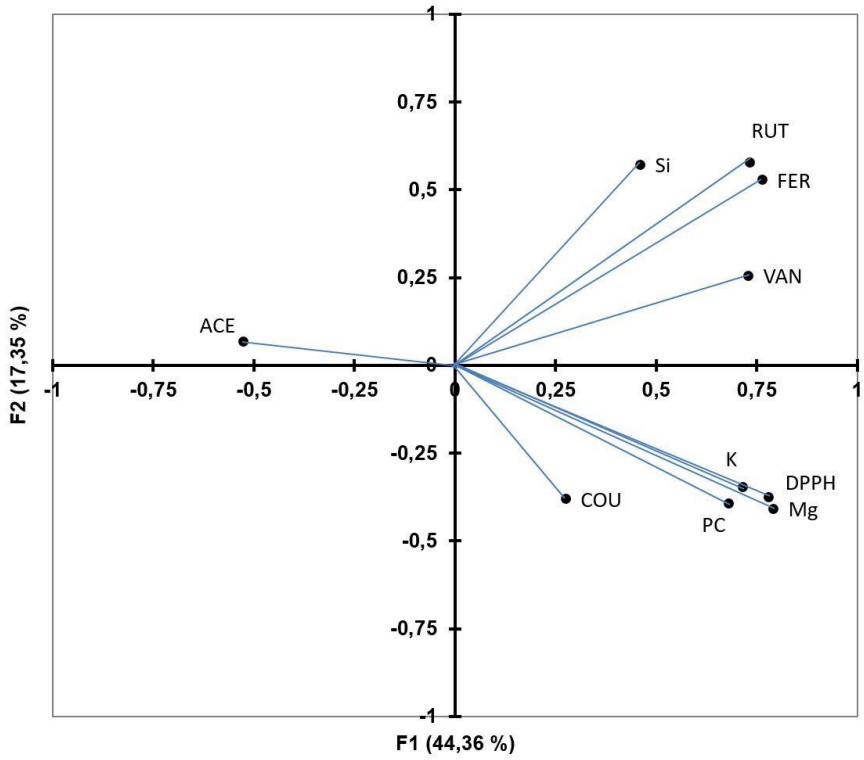

Figure 1. Principal component analysis biplot of principal components F1 and F2 showing chemical parameters' distribution: acetic acid (ACE), coumaric acid (COU), protein content (PC), DPPH radical scavenging capacity (DPPH), concentrations of potassium (K), magnesium (Mg), silicon (Si), vanillic acid (VAN), rutin (RUT), and ferulic acid (FER).

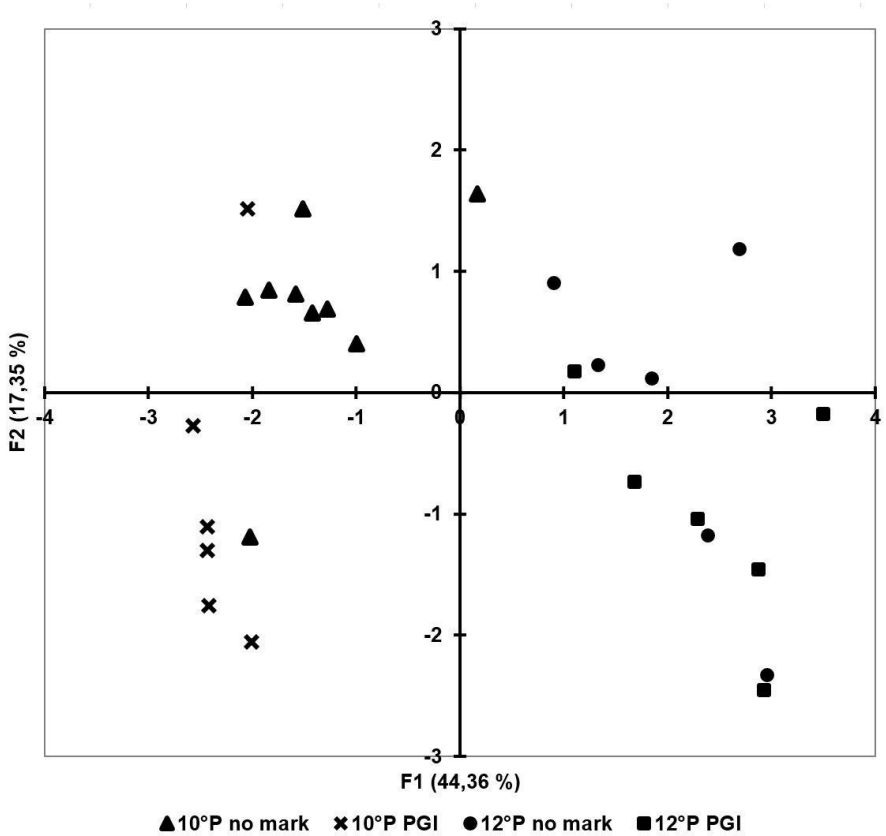

Figure 2. Principal component analysis scores plot of principal components F1 and F2 showing clustering of Czech beer samples.

Conclusion

The present work provided insights into the chemical composition of two main types of Czech beer, characterised by 10°P and 12°P. The results showed significant differences in chemical composition between 10°P and 12°P beers. These variations could be attributed to the use of different amounts and types of raw materials during the brewing process, or through post-fermentation treatments. When comparing 10°P and 12°P beers, with and without the PGI, non-PGI beers were comparable to PGI "Czech Beer" in the studied parameters. This was particularly pronounced in the case of 12°P beers. While the beers without the PGI analysed in this study exhibited similar quality to those with the PGI, the PGI "Czech Beer" application remains important in the international market. The PGI "Czech Beer" serves as a mark of reliability that customers worldwide can depend on to ensure the product meets defined quality standards that are achieved through traditional production methods, and the use of specific raw materials from the country of origin.

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