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STUDY OF THE INFLUENCE OF BREWING WATER ON SELECTED ANALYTES IN BEER

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ABSTRACT

Brewing water is one of the basic raw materials for beer production and knowledge of its composition and pH is essential for the proper conduct of the entire brewing process. In this study, it was observed how the composition of water influences OG values, content of B vitamins, organic acids and iso-α-acids. For brewing, synthetic water was prepared by adding chemicals to deionized water. Models of hard (pH 8.47 ±0.08) and soft (pH 7.68 ±0.23) synthetic water were used for brewing pale bottom-fermented lager beers. Samples of wort, hopped wort, young beer and beer were collected during beer production. HPLC-DAD was used for B vitamins and iso-α-bitter acids quantification. Determination of organic acids was done by ion chromatography with conductivity detector. Obtained data were statistically processed with ANOVA (Analysis of Variance) and interval of confidence was set to 95%. According to the statistical analysis, water composition affects analytes content during beer production and in the final product. Hard water seemed to be a better extraction buffer and its composition (pH) positively affected some processes during brewing technology. One of them was obtaining higher OG values compared to soft water. The beer made from hard water also contained more B vitamins. Composition of brewing water had no influence neither on concentration of organic acids nor on iso-α-acids in conditions of homebrewing.

Keywords: brewing water; homebrewing; B vitamins; organic acids; iso-α-acids

INTRODUCTION

Beer is composed of about 94% of water, so water becomes an essential, but often neglected ingredient in beer production **(Comrie, 1967)**. Water has a significant effect on the chemical and sensory characteristics of beer. Therefore, knowledge of brewing water composition (liquor) is important for breweries.

However, the water must accomplish certain parameters to be used in brewing. The liquor requirements may be grouped as „aesthetic“ (colour, turbidity, odour and taste), microbiological standards (particularly the absence of pathogens), the levels of organic and inorganic materials that are in solution and the presence of radioactive materials **(Briggs et al., 2004)**.

Discussions of brewing water composition often involve the total hardness. The total hardness is defined as the sum of all alkaline-earth ions (calcium, magnesium, strontium and barium ions) **(Kadlec, 2002; Eßlinger, 2009**). It is divided in carbonate and non-carbonate hardness. Common counter ions for non-carbonate (permanent) hardness are sulfate, nitrate and chloride and these remains in solution when the water is boiled **(Briggs et al., 2004; Eßlinger, 2009)**. Carbonate or temporary hardness is caused chiefly by calcium and magnesium bicarbonates and is so-called because if the water is boiled the bicarbonate is converted to the carbonate, which precipitates leaving the clarified water “softened” **(Kadlec, 2002; Briggs et al., 2004)**. Soft water contains low concentrations of dissolved salts, particularly salts of calcium and salts of magnesium. Hard water contains high concentrations of salts, usually mainly calcium bicarbonate or calcium sulphate. The distinction is important if the liquor is to be used for mashing or, even more, for sparging **(Briggs et al., 2004)**. Calcium and magnesium salts are predominant elements in water **(Kadlec, 2002)**.

Other important parameters, which correlate with water hardness are pH and ionic strength **(Basařová et al., 2010)**. Apart from the legal requirements, additional quality criteria for brewing water need to be complied with, since water ions influence the pH value of mash, wort and beer, and thus enzymatic and non-enzymatic reactions. Consequently, these have a considerable influence on the acidity. Hydrogen carbonate ions count as acid destroying since they lead to an increase in the pH value. Calcium and magnesium ions are acidity supporting and lead to a pH decrease of the mash **(Eßlinger, 2009)**.

The dissolved salts are present in water at low concentrations, but significantly affect the sensory qualities of beer, enzymatic activity during mashing and regulate processes during boiling, cooling and

fermentation of the wort **(Comrie, 1967)**. For example, calcium ions serve several important functions in brewing. They stabilize the enzyme α-amylase (approximately 80 °C and higher temperatures cause inactivation of enzyme) during mashing and they interact with phosphate, phytate, peptides and proteins in the mash and during the copper boil, the pH values of the mash and the wort are usefully reduced. Ions in beer can influence its flavour and calcium ions in particular influence the mashing process **(Briggs et al., 2004; Ganbaatar et al., 2015)**.

Water hardness is important in assessing the quality of water used for brewing. The composition of water is conditioned by the place of occurrence. Historically, different regions became famous for particular types of beer and in part these beer types were defined by the waters available for brewing. One of them is Pilsner water, which is well-known as a very soft water with small proportion of inorganic compounds. It is suitable for pale and delicate lagers. Burton-on-Trent, with its extremely hard water, rich in calcium sulphate, is famous for its pale ales. Compared to that, Munich is well-known for its dark lagers **(Rudin, 1976; Basařová et al., 2010; Kábelová-Ficová et al., 2017)**.

It is now usual for breweries to adjust the composition of the brewing water they use **(Comrie, 1967; Briggs et al., 1981; Briggs et al., 2004)**. However, homebrewers are not used to treat brewing water, but if they do, they are often limited with availability of technology. Treatments may reduce levels of organic compounds in solution or adjust the ionic composition of the liquor **(Briggs et al., 2004)**.

Sodium chloride may be added to brewing liquors (75 – 150 $mg.L^{-1}$) to enhance "palate-fullness" and a certain sweetness. Sometimes potassium chloride is added instead, at low concentrations, to achieve a less sour flavour **(Briggs et al., 2004)**.

The aim of this study is to assess the impact of the composition of the brewing water on selected analytes in the technology of beer. For experiments, Pilsner type beers of soft and hard synthetic water were brewed, and samples of wort, hopped wort, young beer and beer were taken during production. Subsequently, the samples were analysed. HPLC was used for the assay. OG values were determined by refractometer and pH values were determined by pH meter. The assessment of differences between soft and hard water was provided by the statistical method of analysis of variance (ANOVA).

Scientific hypothesis

Precedent published studies dealt with composition of water and its effect on brewing. The aim of this study was to find out, whether composition of brewing water significantly influences selected analytes during beer brewing and in the beer in conditions of homebrewing.

MATERIAL AND METHODOLOGY

Pilsner type beers were brewed from soft and hard synthetic water and analysed as follows.

Synthetic water preparation and beer production

Synthetic water was prepared according to **Smith, Davison, and Hamilton-Taylor (2002)** by adding selected compounds to distilled water. Thus, soft and hard model water was created.

For study of the influence of brewing water on selected analytes, beer was made from soft and hard synthetic model water in three replicates. These were the pale bottom-fermented beers also called lagers. The raw materials were pale Pilsner barley malt (Malt house Bernard, Rajhrad), Sládek hop pellets with alpha acid content of 8.08% and Saflager S-23 yeasts.

Milled barley malt was mixed with brewing water at 38 °C and then an infusion method was chosen for mashing process. Evaporated water was compensated with distilled water. The values of original gravity (OG) and pH were measured in wort after lautering. Then it was continued with wort boiling for 90 min. After this process, whirlpooling of wort was done. Also the values of OG and pH were measured. After that wort was cooled down and then it was inoculated and aerated and stored at 14 °C. The degree of attenuation was checked by measuring the OG value to confirm that yeasts completed fermentation. The OG value was in range of 7.0 – 7.3%. Values of OG and pH were measured in the samples of young beer. Then bottled beer was stored at 7 °C. The secondary fermentation and maturing last for three weeks.

Measurement of basic quantitative parameters of tested beers

The OG values were measured refractometrically (A. Krüss Optronic GmbH, Hamburg, Germany). The pH of synthetic water was measured with pH meter (WTW, Germany).

Determination of B group vitamins by HPLC analysis

The samples of wort, hopped wort, young beer and beer were degassed by sonication (Ultrasonic Compact cleaner PS03000A 2.5 L, PowerSonic s r.o.) and then diluted with distilled water. All samples were analysed by HPLC-DAD (Agilent 1260 Infinity, Agilent Technologies, USA) to determine the quantity of B vitamins. Specifically, it was focused on riboflavin, niacin, pyridoxine and cobalamin. B vitamins were separated on Polar C18 column (150 mm x 3.0 mm; particle size 2.6 μm) with set temperature to 40 °C. The mobile phase consisted of 10 mM ammonium formate (Sigma-Aldrich Inc., USA) and 0.1% solution of formic acid (solvent A) (Sigma-Aldrich Inc., USA) and acetonitrile (Sigma-Aldrich Inc., Germany) and 0.1% solution of formic acid (solvent B) (Sigma-Aldrich Inc., USA). Gradient elution was used for the analysis. The gradient was as follows: 0 min, 100% A; 12 min, 40% A; 15.10 min, 100% A and then held for another 7 min. The samples were detected with DAD detector (260 nm, 270 nm, 271 nm, 292 nm, 360 nm). The data were collected by the Agilent 1260 Infinity chromatographic data system.

Determination of organic acids by IC analysis

Ion chromatography (850 Professional IC, Metrohm, Switzerland) was used to determine lactic and acetic acid in the samples of wort, hopped wort, young beer and beer. Each sample was degassed by sonication (Ultrasonic Compact cleaner PS03000A 2.5 L, PowerSonic s r.o.) for 20 minutes. Then the samples were filtrated using filters

with 0.45 μm pore size (Labicom, Czech Republic) and diluted with distilled water in proportion 1:1.

Organic acids were separated on Metrosep Organic Acids column (250 mm x 7.8 mm; particle size 9 μm) with temperature set to 30 °C. The mobile phase was 0.5 mmol perchloric acid (Sigma-Aldrich Inc., USA). Isocratic elution was used and the flow of mobile phase was 0.6 $mL.min^{-1}$. The analytes were detected with conductivity detector.

Analysis of hop bitter acids

Extraction of iso-α-acids

Degassed samples of hopped wort, young beer and beer (10 mL) were transferred into a 50 mL centrifuge tube, then sample was acidified with orthophosphoric acid (0.5 mL) (Lach-Ner, s.r.o., Czech Republic). The mixture was extracted into isooctane (10 mL) (Sigma-Aldrich Inc., USA). Mixture was vortexed for 1 minute and methanol (1 mL) (Sigma-Aldrich Inc., USA) was added. Thereafter, the two-layered solution was centrifuged for 5 minutes at 2,000 rpm (Z 36 HK, HERMLE Labortechnik GmbH). Then 4 mL of supernatant was collected into a glass tube, evaporated under N_2 gas and redissolved in methanol (2 mL) (Sigma-Aldrich Inc., USA) to give the HPLC sample.

Determination of iso-α-acids by HPLC analysis

After filtration (filters with 0.45 μm pore size) (Labicom, Czech Republic), samples were subjected to quantitative HPLC analysis (Agilent 1260 Infinity, Agilent Technologies, USA). Iso-α-acid content (isohumulone, isocohumulone and isoadhumulone) in the samples were measured simultaneously using ICS-I4 as a calibration standard (Labor Veritas AG, Switzerland). Iso-α-acids were separated on Poroshell 120 EC – C18 column (4.6 mm x 50 mm; particle size 2.7 μm) with temperature set to 40 °C. Isocratic elution was chosen. The mobile phase consisted of 0.1% solution of H_3PO_4 (Sigma-Aldrich Inc., Germany) and 0.2 mM Na_2EDTA (solvent A) (Sigma-Aldrich Inc., France) and acetonitrile (solvent B) (Sigma-Aldrich Inc., Germany) in proportion A:B = 35:65. Flow of mobile phase was 1 $mL.min^{-1}$. The analytes were detected with DAD detector (270 nm). The data were collected by the Agilent 1260 Infinity chromatographic data system.

Statistic analysis

Data analysis and statistical evaluation was carried out by software XLSTAT (Addinsoft, USA). Influence of the brewing water on the selected parameters of beer was evaluated by Analysis of Variance (ANOVA). ANOVA was set to a confidence interval of 95%.

RESULTS AND DISCUSSION

Selected quantitative beer indicators

First, the pH of prepared synthetic model water was measured. The average pH value of soft water was 7.68 ±0.23, the average pH value of hard water was 8.47 ±0.08. Compared to theoretical pH, the measured values were lower. The theoretical model of soft water pH was 7.83 and 8.49 hard water **(Smith, Davison and Hamilton-Taylor, 2002)**.

Figure 1 shows difference between pH of soft and hard water during mashing. During mashing, the pH decreases due to malt enzymes activity, which releases phosphates from nucleic acids. In the process of wort boiling, the wort acidity is increased by precipitation of phosphates in the presence of calcium and magnesium ions. Hop bitter acids and Maillard reaction products further contribute to pH reduction. When fermenting, the pH declines by the activity of yeasts that consume amino acids and produce organic acids. The pH also changes because of presence of the carbon dioxide, which dissolves in the solution **(Basařová et al., 2010)**.

OG (original wort extract) and ABV (Alcohol by Volume) were selected as the basic quantitative indicators for beer brewing. The extract of the original wort was used to express the total carbohydrate content presented in the medium. Differences in beers brewed from different kind of synthetic water were observed. Both, original gravity and concentration of alcohol were statistically different. The mean value of wort OG prepared by using soft water was 12.6 ±0.1%, while wort OG of hard water was 13.05 ±0.05% (see Figure 2). Higher yields in hard water are probably due to higher amounts of Mg^{2+} and Ca^{2+} ions, which stabilize α-amylases and increase its activity **(Karbassi and Saboury, 2000; Saboury, Ghasemi and Umar Dahot, 2005)**. After wort boiling, the OG slightly increased, probably due to the extraction of chemical compounds in hop. A large decline occurred during fermentation when yeasts utilized carbohydrates. A small drop occurred during secondary fermentation and maturing in bottles because yeasts largely depleted the substrate and was no longer as vital as at the beginning. The process of fermentation of all samples is shown in Figure 3. As the yeast assimilated the substrate, the OG also declined and the ethanol concentration in the medium grew as expected. Complete fermentation of experimental beers took 70 hours.

Quantification of selected B vitamins

Determination of B2 (riboflavin), B3 (niacin), B6 (pyridoxine), B12 (cyanocobalamin) vitamins were done to assess the influence of the two different kind of experimental brewing waters on content of B vitamins. The concentration of the last two mentioned vitamins was below the detection limit in all samples. The results of the analysis of the determined B vitamins are shown in Table 1 and Table 2.

Statistically significant differences between hard and soft water were found in wort (p = 0.0007, F = 92.214). Hard water seemed to be a better extraction agent due to different pH and ionic strength. Content of B3 vitamin changed during the brewing. In the presence of yeast, the level of B3 vitamin declined rapidly as yeast used vitamin in the wort in biochemical processes and nicotinic acid synthesis did not occur **(Basařová et al., 2010)**. Relatively small, but significant difference in concentration of B3 vitamin was found in the final product (p = 0.0448, F = 8.3243). A slightly higher average concentration of vitamin B3 was determined in beer from hard water. This phenomenon is probably caused by releasing cell content during yeast autolysis.

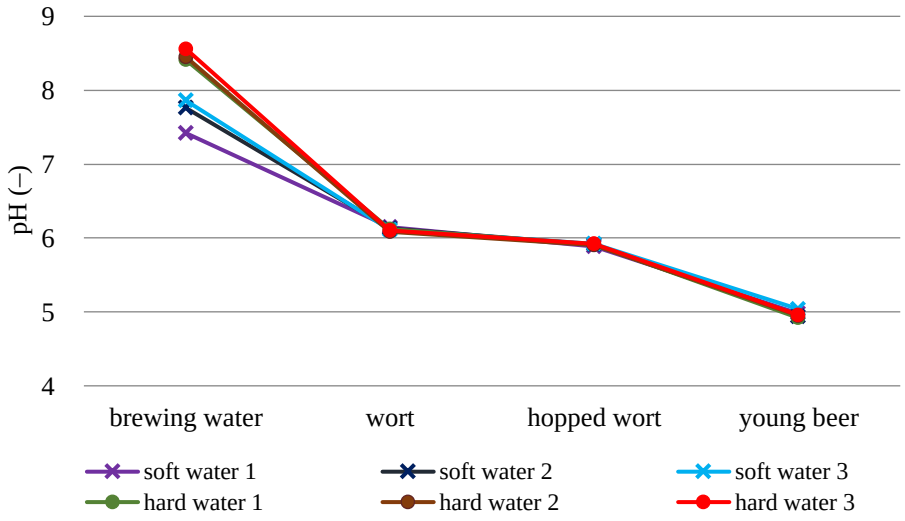

Figure 1 Changes of synthetic water pH during beer production.

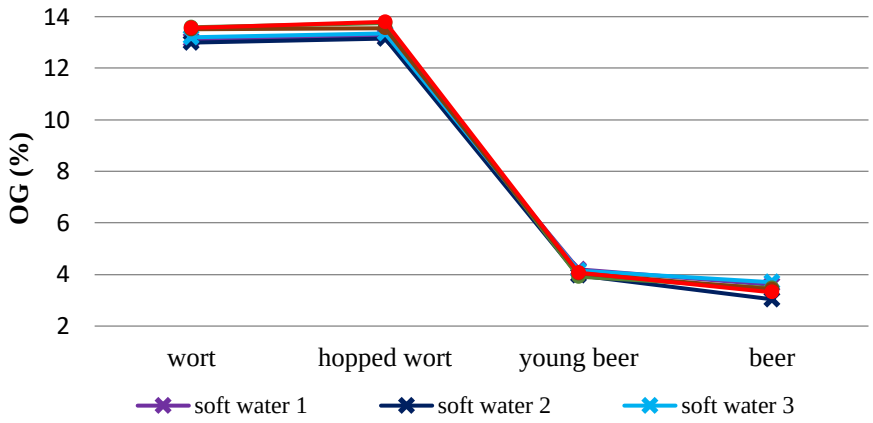

Figure 2 Changes of OG in individual phases of beer production.

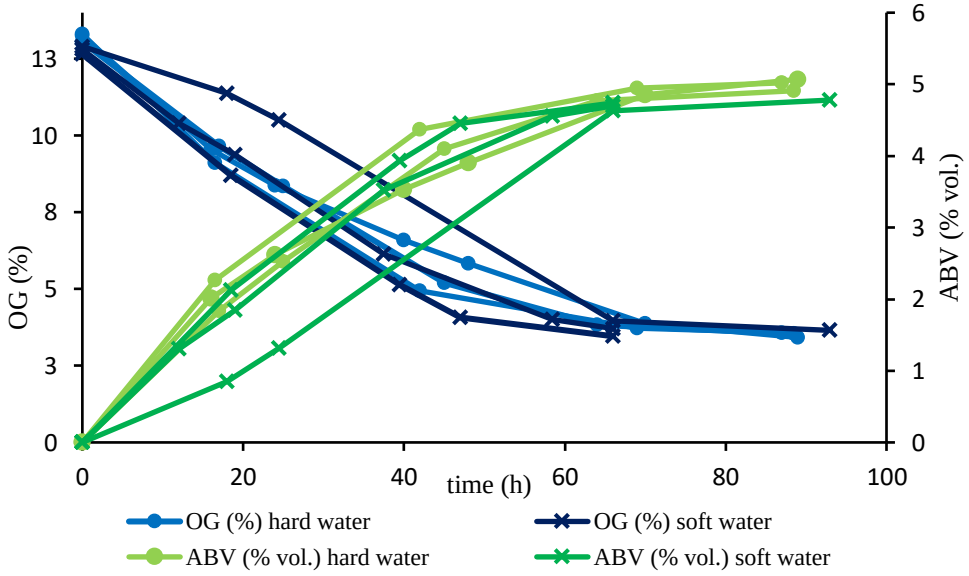

Figure 3 OG and ABV changes during primary fermentation.

Table 1 Quantification of B3 vitamin (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	31.68	30.07	33.18	0.0007	92.2144
	Hard	40.85	40.25	41.36		
Hopped wort	Soft	36.13	32.92	41.56	0.0674	6.2091
	Hard	43.05	42.14	43.97		
Young beer	Soft	8.97	6.70	10.59	0.7838	0.0861
	Hard	9.34	8.54	10.11		
Beer	Soft	8.99	7.97	10.02	0.0448	8.3243
	Hard	10.97	10.47	11.63		

Note: * All samples were made in triplicates.

Table 2 Quantification of B2 vitamin (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	0.90	0.84	0.93	0.0385	9.2210
	Hard	1.00	0.96	1.01		
Hopped wort	Soft	1.09	0.97	1.18	0.5513	0.4221
	Hard	1.14	1.11	1.16		
Young beer	Soft	0.29	0.27	0.33	0.0008	83.2461
	Hard	0.57	0.53	0.62		
Beer	Soft	0.77	0.70	0.90	0.3008	1.4099
	Hard	1.02	0.62	1.25		

Note: * All samples were made in triplicates.

Table 3 Quantification of acetic acid (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	34.00	33.64	34.18	0.7063	0.1640
	Hard	34.21	33.26	34.87		
Hopped wort	Soft	34.92	33.83	35.87	0.0998	4.5510
	Hard	36.51	35.61	37.02		
Young beer	Soft	87.91	86.40	89.94	0.0786	5.5162
	Hard	111.25	93.26	127.32		
Beer	Soft	122.42	105.56	143.49	0.5623	0.3982
	Hard	115.28	112.72	118.97		

Note: * All samples were made in triplicates.

Table 4 Quantification of lactic acid (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	94.49	93.35	95.95	0.6203	0.2874
	Hard	93.87	92.55	95.48		
Hopped wort	Soft	94.74	93.86	95.92	0.1181	3.9418
	Hard	98.04	95.33	100.67		
Young beer	Soft	225.55	179.34	271.00	0.0988	4.5894
	Hard	287.48	266.54	306.74		
Beer	Soft	300.06	299.22	304.71	0.1020	4.4690
	Hard	298.37	288.99	299.44		

Note: * All samples were made in triplicates.

Table 5 Quantification of isocohumulone (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	1.04	0.93	1.14	0.5836	0.3545
	Hard	1.07	1.05	1.10		
Hopped wort	Soft	0.57	0.45	0.75	0.6376	0.2590
	Hard	0.62	0.54	0.67		
Young beer	Soft	0.40	0.26	0.64	0.5619	0.3991
	Hard	0.49	0.35	0.59		

Note: * All samples were made in triplicates.

Table 6 Quantification of isohumulone (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	2.23	1.97	2.49	0.7584	0.1085
	Hard	2.29	2.15	2.41		
Hopped wort	Soft	0.74	0.42	1.13	0.9758	0.001
	Hard	0.75	0.52	0.94		
Young beer	Soft	0.50	0.31	0.76	0.7393	0.1273
	Hard	0.57	0.34	0.79		

Note: * All samples were made in triplicates.

Table 7 Quantification of isoadhumulone (F_{krit} = 7.71).

Type of sample	Type of water	Mean (mg.L^{-1})	Min (mg.L^{-1})	Max (mg.L^{-1})	*p*	F
Wort	Soft	44.63	39.83	50.44	0.8564	0.0372
	Hard	45.26	43.22	46.96		
Hopped wort	Soft	17.68	11.26	25.55	0.9472	0.0050
	Hard	17.35	13.33	20.68		
Young beer	Soft	10.36	6.02	17.15	0.5314	0.4681
	Hard	13.25	8.59	16.90		

Note: * All samples were made in triplicates.

Usual content of niacin in beer is around 5 mg.L^{-1} **(Basařová et al., 2010)**. The measured values are higher in comparison with the literature, approximately 8 – 12 mg.L^{-1}. High vitamin content can be caused by no further treatment after beer bottling, unlike commercial beers, which are filtered and often pasteurized.

Very similar trend was observed for B2 vitamin. Statistically significant differences were found in wort (p = 0.0385, F = 9.2210) and young beer (p = 0.0008, F = 83.2461) (shown in Table 2). Riboflavin was better extracted from barley malt during mashing in hard brewing water than in soft. This could be due to different pH of the water. Riboflavin is a thermostable vitamin, there were observed no loss of the vitamin in the process of wort boiling. During fermentation there was a certain decrease in vitamin content because riboflavin participates in many biochemical processes **(Hucker, Wakeling, Vriesekoop, 2016)**. The greatest difference was observed in soft water. In hard water, to thereby prevent high losses probably because riboflavin form stable complexes with Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+} **(Sheraz et al., 2014)**. In beer, the vitamin concentration was probably increased by the release of yeast autolysis **(Hucker, Wakeling, Vriesekoop, 2011)**.

Usual content of riboflavin in beer is approximately 0.25 mg.L^{-1} **(Olšovská, Jurková and Čejka, 2012)**. When comparing the values and literature the B2 vitamin concentration is several times higher – 0.6 to 1.2 mg.L^{-1}. Again, it is probably the reason for omitting post fermentation adjustments.

Quantification of organic acids

Determination of organic acids was done to assess the influence of soft and hard experimental brewing waters. Retention time of lactic acid was 11.17 min, acetic acid 14.22 min. The results are shown in Table 3 and Table 4.

The results have shown that there are no statistically significant differences in content of acetic acid in beer made from soft and hard brewing water (Table 3).

The content of acetic acid increases rapidly during fermentation **(South, 1996)**. The greatest changes between samples made from soft and hard brewing water were found in young beer, though Analysis of Variance did not prove statistically significant differences. This difference was probably due to a divergent ratio of minerals in hard and soft brewing water, which can either positively or negatively affects the level of organic acid synthesis.

Concentration of acetic acid in beer brewed from soft and hard brewing water varied from 106 to 144 mg.L^{-1}, whereas **Vontrobová et al. (2017); Coote and Kirsop (1974); Walker (1998); Zhang, Jia and Zhang, (2012)** indicate values ranging from 30 to 200 mg.L^{-1}.

Lactic acid consists of two isomers. D-lactate, which comes from malt and the content may vary during mashing, and L-lactate, which indicates bacterial activity. The D and L isomers of this acid have been reported in wort and beer on a number of occasions **(Coote and Kirsop, 1974)**. Lactate was excreted throughout the period of sugar utilization **(Coote and Kirsop, 1974; Whiting, 1976)**.

The results of determination of lactic acid are shown in Table 4. The difference in composition of brewing water had no influence on content of lactic acid. During wort boiling, it was observed no decline of acid, concentration slightly increased in hard brewing water. It could be possibly related with extraction of organic acids from hops. High production of acid occurred during fermentation. Higher amounts of lactic acid were obtained in young beer made from hard brewing water (p = 0.098 8, F = 4.589 4). On the contrary, higher concentration was determined in beer prepared from soft water (p = 0.102 0, F = 4.469 0), while the concentration in beer made from hard water remained the same.

Experimental beers content high amounts of lactic acid, the concentration was ranging between 289 – 305 mg.L^{-1}. **(Coote and Kirsop, 1974; Whiting, 1976)** present big range of lactic acid concentration in beer. Specifically, this span varies from 37 to 233 mg.L^{-1} in lagers, ales 44 – 276 mg.L^{-1} and dark strong beers 276 – 292 mg.L^{-1}.

Lactic acid bacteria (LAB) may be the reason of high concentrations of lactic acid. They may proliferate in wort, hopped wort and even during fermentation. They survive in pH 4 – 7. Optimal growth temperature is 20 – 30 °C, but psychrophilic strains occur, which may grow in conditions of low temperatures during fermentation and maturing of beer **(Basařová et al., 2010)**.

Quantification of iso-alpha-acid

The results of iso-α-acids analysis are in Table 5, Table 6 and in Table 7. The retention time of isocohumulone was 7.481 min, isohumulone 9.610 min and isoadhumulone 10.256 min.

Impact of brewing water composition was studied on the most represented analogues of iso-α-acids – isohumulone, isocohumulone and isoadhumulone. Analysis of variance did not prove statistically significant differences neither in soft nor hard brewing water. Isomers of α-acids are unstable and during beer production its concentration declined rapidly. Of the total amount of bitter compounds 30% at most will end in beer, whereas approximately 20% remains in the spent hops, 20 – 30% is captured in waste and 20 – 30% is captured in the head of foam on the top of the fermentation. Isomerization is also affected by time of wort boiling and OG value **(Basařová et al., 2010)**.

CONCLUSION

In conclusion, brewing water significantly influences some selected parameters. In the final product – beer – it was found out that hard brewing water is better for higher yields of OG values (OG value equals 13.05 ±0.05%) than soft water (OG value 12.6 ±0.1%).

Brewing water also affected the content of riboflavin and niacin. Statistically significant difference in concentration of B3 vitamin appeared between hard and soft water in wort (p = 0.0007, F = 92.214) because hard water was a better extraction agent due to different pH and ionic strength. Another significant difference in concentration of B3 vitamin was found in the stabilized beer (p = 0.0448, F = 8.3243). A higher concentration of B3 vitamin was determined in beer from hard water. It is probably caused by yeast autolysis when the cell content is released into solution. Very similar trend was observed for B2 vitamin. Statistically significant differences were found in wort (p = 0.0385, F = 9.2210) and young beer (p = 0.0008, F = 83.2461).

Unlike statistical analysis did not prove an effect on content of organic acids or iso-α-acids.

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