A Contribution to Analysis of "Czech Beer" Authenticity

S. OBRUČA[1], I. MÁROVÁ[1], K. PAŘILOVÁ[1], L. MÜLLER[1], Z. ZDRÁHAL[2] and R. MIKULÍKOVÁ[3]*

[1]Faculty of Chemistry, Brno University of Technology, 612 00 Brno, Czech Republic; [2]Laboratory of Functional Genomics and Proteomics, Faculty of Science, Masaryk University of Brno, 625 00 Brno, Czech Republic; [3]Institute of Beer Research, Malting Institute, 614 00 Brno, Czech Republic, E-mail: xcobruca@fch.vutbr.cz

Abstract: Total benefit effect of beer is a result of many individual contributions of natural substances present in such complicated biological material. Recently, regional mark "Czech beer" was obtained by EC. This beer is processed by traditional technology using only recommended brewery materials. Presented work is focused on proteomic and metabolomic analysis of some characteristic active substances specific for "Czech beer". Eight beer samples obtained from retail chain and/or from breweries were enrolled into this study. Polypeptides and proteins that influence beer sensory properties originated mainly from malt. The results of our preliminary study indicate that the main protein fractions in most of beers are protein Z, LTP1 and hordein/glutelin fragments. 2D analyses of "Czech beer" differed in several spots when compared with beer made by other technology. Further, individual beer phenolics originated from malt and hop were analysed by HPLC/UV-VIS and LC/MS. Individual beers differed in distribution as well as in content of phenolics. Hence, some phenolic derivatives seem to be useful as potential authenticity markers.

Keywords: hop; malt; phenolics; natural substance; Czech beer; authenticity

INTRODUCTION

The healthful and nutritive properties of beer have been recognised by the medical profession for thousands of years. Clinical and statistical evidence and laboratory studies have shown that active substances in beer could influence immune system, block cancer formation, protect against coronary disease and even prolong life. Total beneficial effect of beer and malt samples is a result of many individual contributions of natural substances present in beer (Zimmermann & Galensa 2007).

Within the last few years, development of modern instrumental analytical methods has gained increasing importance in authenticity control of food and food ingredients. The characterisation of beer samples has a lot of interest because their composition can affect the taste and stability of beer as well as consumer health. Many substances could contribute to final taste of beer. To the most important probably belong proteins (Perrocheau *et al.* 2005; Silva *et al.* 2006) and phenolic substances (Silva *et al.* 2006).

The aim of this pilot study is to compare composition and to find specific and/or characteristic components in several kinds of beer.

MATERIALS AND METHODS

Materials. Seven samples of lager beer were gained from breweries (Pilsner Urquel) and retail chain (Starobrno, Heineken, Zlatopramen, Velkopopovický Kozel, Braník).

Antioxidant analysis. Total phenolics were analysed colometrically with Folin-Ciocalteu reagent (750 nm). Total flavonoid content was analysed

colometrically with $NaNO_2$ + $AlCl_3$ (510 nm). Total antioxidant capacity (TAS) was measured by Randox kit.

Individual phenolic substances were analysed by RP-HPLC/UV-VIS method. Using external standards calibration of (-)catechin, catechin gallate, chlorogenic acid, epicatechin, morin, quercetin and rutin was done. Samples (20 µl) were injected into the RP-18 column (Biospher PSI 200 C18, 7 ìm, 150 mm × 4.6 mm). Mobile phases were methanol/water (55:45) for catechins and methanol/acetonitrile/water + 1% phosphoric acid (20:30:50) for flavonoids analysis. The flow rate was maintained at 0.75 ml/min, analysis was performed at 30°C.

Identification of individual flavonoids and catechins were performed by on-line LC/MS/ESI analysis (Mass spectrometer LCQ Advantage Max). Samples of beer were mixed with 5-times higher amount of 2% HCl and extracted by SPE (Amid-2 column). Separation and detection of phenolics were performed using followed conditions: Restek C18 Ultra aqueous column heated on 30°C, gradient elution using 1% acetic acid:acetonitril in range 60–45:40–55 with flow of mobile phase 0.4 ml/min, UV-VIS detection (280 nm and/or 370 nm), MS tune file on chlorogenic acid (negative ion mode). Standard compounds as well as food samples were determined by MS full scan and/or MS/MS full scan mode.

Protein analysis. 1D PAGE-SDS electrophoresis of proteins was carried out by common procedure using 15% and 17.5% polyacrylamide gels. Proteins were stained by Coomassie Blue and by silver staining. For comparison, microfluidic technique using 1D Experion system (BioRad) and P260 chips was used for yeast protein analysis too. 2D electrophoresis of proteins was optimised in cooperation with Laboratory of Functional Genomics and Proteomics, Faculty of Science, Masaryk University of Brno. After optimisation of separation conditions proteomes from lyophilised beer samples were analysed. Quantitative analysis were done using BioRad Laboratories 2D software. Identification of some spots were done using LC-MS/MS.

RESULTS AND DISCUSSION

The aim of presented pilot study is to determine characteristic components of "Czech beer". One of the main goals is separation and analysis of specific proteins and peptide fragments. Further aim is qualitative and quantitative analysis of individual phenolics in "Czech beer" by chromatographic techniques. All analyses are performed in several kinds of "Czech beer" in comparison with beers processed by different technology.

Barley and malt proteins are relatively well-documented and their analysis belongs to commercially used tests for barley variety authenticity. Beer proteome has been not studied in details. In this work main protein fractions (40 kDa, lower than 8–10 kDa peptides) were determined in lyophilised beer samples using 1D electrophoresis and microfluidic electrophoresis. Pilot 2D electrophoresis analysis of some beer samples were done mainly for optimisation of analysis conditions. 2D gels were obtained using purified protein extracts. To remove interference of protein Z in 2D GE repeat extraction and chromatography procedures were used. Quantitative analysis was done using BioRad Laboratories 2D software. Preliminary identification of about 20 spots was done using LC-MS/MS. The main protein fractions in most of beers are protein Z, LTP1 and hordein/glutelin fragments.

Table 1. Total antioxidant capacity, total phenolic and flavonoids content of analysed beers

Beer sample	Total phenolics (mg/l)	Total flavonoids (mgl)	TAS (mmol/l)
Kozel	181.23 ± 17.52	71.20 ± 6.12	2.94 ± 0.15
Starobrno	182.64 ± 16.15	68.36 ± 5.33	2.99 ± 0.11
Heineken	156.49 ± 11.23	53.49 ± 6.52	2.74 ± 0.10
Sample No. 49	201.15 ± 12.85	76.80 ± 3.54	3.13 ± 0.12
Sample No. 50	197.30 ± 15.16	69.89 ± 2.85	3.01 ± 0.13
Zlatopramen	178.15 ± 8.83	65.43 ± 3.64	2.86 ± 0.16
Braník	165.45 ± 11.58	59.87 ± 4.12	2.72 ± 0.10

Table 2. Identification of individual phenolics (LC-ESI/MS)

		MW	Velkopovický Kozel	Starobrno	Heineken	SampleNo. 49	Sample No. 50	Zlatopramen	Braník
1		194	–	+	–	–	–	+	–
2	gallic acid	170	–	+	–	–	+	-	–
3	4,6-di-*O*-methylchalkonaringenin × 5,7-di-*O*-methylnaringenin	300	+	+	+	+	+	–	–
4	6-prenylnaringenin × 8-prenylnaringenin	340	+	+	–	–	–	–	–
5		180	+	+	–	+	+	–	–
6	epicatechin	290	–	+	+	+	–	+	+
7		330	–	+	–	–	–	–	–
8	catechin	290	+	+	+	+	+	+	+
9	florisin	496	+	+	+	–	–	–	–
10	caffeic acid	180	–	+	+	+	+	–	–
11	5,7-di-*O*-methyl-8-prenylnaringenin	368	+	+	+	+	+	–	+
12	*p*-coumaric acid	164	+	-	+	+	+	+	+
13	ferulic acid	194	+	+	+	+	+	+	+
14		164	+	-	+	+	+	–	+
15		300	–	+	-	–	–	–	–
16	xanthogalenol	354	–	+	-	–	–	–	–
17		330	+	+	+	–	+	+	+
18	prenylxanthohumol	422	+	–	–	–	–	–	–
19		368	+	+	+	–	–	–	–
20	4-*O*-methylxanthohumol	368	+	+	+	+	+	+	+
21		352	–	+	–	+	–	+	-
22	xantohumol	354	+	+	+	+	+	+	+
23	isoxanthohumol	300	+	+	–	–	+	–	+
24	rutin	610	+	+	+	+	+	+	+

MW – molecular weight; – not detected; + detected

2D analyses of Czech beer differed in several spots as compared with beer made by other technology. Nevertheless, further analysis will be needed for detailed identification of proteins specific for individual beers.

In further part of this work, levels of total phenolics, total flavonoids and TAS in 7 different kind of lager beer were analysed. These beers were made by different technology and obtained from retail chain as well as directly from Pilsner Urquel brewery (samples No. 49, No.50). The levels of above mentioned parameters in most of beer samples were quite similar (Table 2). Nevertheless, significantly lower phenolic and flavonoid levels were observed in Heineken beer, low values were found also in Braník beer.

Individual phenolics were analysed using LC-ESI/MS in negative mode. Up this now, we have found 24 individual phenolics, 15 of them were identified according their molecular weight and retention time. The identification was verified using commercial available external standards. All

beers contained catechin, ferulic acid, 4-*O*-methylxanthohumol, xanthohumol and rutin. Most of beers (at least 5 of 7 samples) also contained 5,7-di-*O*-methylnaringenin, epicatechin, 5,7-di-*O*-methyl-8-prenylnaringenin, *p*-coumaric acid (12), its derivate (Mr 164) and also two non-identified polyphenols (17 – Mr 330; 23 – Mr 300) (Table 2). Three other phenolic compounds, isoxanthohumol and two non-modified phenolics (5 – Mr 180, 21 – Mr 352) were not detected in foreign beers and, oppositely, there were found in beers produced using traditional Czech technology. It is necessary to identify unknown phenolic derivatives.

Based on our preliminary results it can be concluded that particular specific proteins as well as some individual phenolics could be used for verification of beer authenticity.

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