KVASNÝ PRŮMYSL

Kvasny prumysl (2019) 65: 120–126
https://doi.org/10.18832/kp2019.65.120

How does fermentation, filtration and stabilization of beer affect polyphenols with health benefits

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Abstract

Phenolic substances affect the quality of beer and some of them have the health benefit for the consumer. Their content in the final product is influenced by a number of raw material-technological factors. We carried out 200 L pilot brews of pale lager with the different maturation period and similar brews focused on the effect of filter material (depth filtration plates /DFP/) and polyester sulfone membrane /PES/) and beer stabilization with protein and polyphenol sorbents. Flavonoids were determined using liquid chromatography in conjunction with high resolution mass spectrometry (LC-HR/MS) and sample preparation by QuEChERS method. Additionally, total polyphenols and anthocyanogens was measured. The major reduction of both total polyphenols and flavonoids occurred in the first 2–3 weeks of maturation, the longer maturation had no impact on the loss of polyphenols. Filtration of beer with DFP significantly reduced the amount of anthocyanogens, but had no effect on monomeric flavonoid polyphenols. This technique could improve colloidal stability while preserving flavonoids. Conversely, PES membrane filtering greatly reduced the prenylflavonoid content (by 85%), and reduced the amount of flavanols and flavonols at levels comparable to those of PVPP-based polyphenol sorbent (25–35%). Flavonoids in beer could be largely influenced not only by PVPP treatment, but also by membrane filters used for both cold sterilization and primary beer filtration. Protein sorbent stabilization did not affect the content of flavonoids in beer. Decreases in flavonoid glycosides during filtration/stabilization were always lower than those of free flavonoids.

Key words: polyphenols, flavonoids, filtration, colloidal stabilization, beer

1 Introduction

Polyphenol substances can affect beer quality, colloidal and flavor stability (Guido et al, 2007; Aron and Shellhammer 2010, Callemien and Collin 2010). They are a very diversified group of substances, whose individual components differ considerably in their chemical structure and therefore have different reactivity in terms of antioxidant, antiradical and metal chelating abilities and other reactions occurring in beer or living cells. Some simple and more complex polyphenols and their oxidation products are sensory active, affecting the bitterness and astringency of beer (Callemien et al., 2005; Oladokun et al., 2015, McLaughlin et al., 2008).

Polyphenol antioxidants of malt (Guido et al., 2007) and hops (Mikyška et al., 2011) can slow down the sensory aging of beer. Both hops and malt are a source of phenolic substances with potential or proven biological effects. Both raw materials contain phenolic carboxylic acids (e.g. ferulic acid, gallic acid), monomeric and oligomeric flavonoids, in particular flavanol monomers (eg catechin), oligomeric proanthocyanidins and flavonols (eg quercetin).

The role of some polyphenols in the chain of radical reactions may be ambivalent. For example, some authors have shown a positive effect of gallotanins on sensory stability of beer (Aerts et al., 2004), others have demonstrated the prooxidative effect of delphinidine, a monomeric building block of gallotanins (Bamforth, 1999).

A unique group are hop prenylflavonoids with a spectrum of antioxidant, anticancer, estrogenic, antimicrobial

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Published online: 15 August 2019

and other beneficial effects (Karabín et al., 2016). Prenylflavonoids are related to both polyphenols and bitter acid hops (Stevens et al., 1998), which are also biologically active and contribute to the antioxidant stability of beer by chelating iron ions (Wietstock et al., 2016). 8-Prenylnaringenin is probably the most effective phytoestrogen. In common beers, 8-PN concentrations are very low (<50 μg/l), and are considered physiologically insignificant. However, the intestinal bacteria are found to be able to transform the isoxanthohumol present in beer to 8-PN, and the daily intake of phytoestrogen by regular beer consumption can thus rise to a physiologically active level (Possemiers et al. 2005; Possemiers et al., 2006).

Catechins (flavan-3-ol monomers, (epi) catechin, (epi) galocatechin) and proanthocyanidins, flavan-3-ol oligomers and polymers that yield anthocyanidins after acid depolymerization, also known as condensed tannins, are important both in terms of health benefits, occur in plants where they have different physiological and defense functions (Karabin et al., 2016; Lotito et al., 2000, Quinones et al., 2013) and affect haze stability of beer (Aron and Shellhammer, 2010; Callemien and Collin, 2010; Steiner et al., 2010). These compounds are also flavor active (Oladokun et al. 2016).

Flavonols, especially quercetin, myricetin, caempferol and their glycosides, such as rutin (quercetin-O-rutinoside), are considered to be very important plant polyphenol antioxidants (Karabin et al., 2016; Nowak et al., 2014, Quinones et al., 2013), rutin and quercetin are part of the pharmaceuticals and dietary supplements. There is a discussion about the bioavailability of glycosides of flavanols and flavonols, deglycosylation of some glycosides occurs already in the mouth and continues in the small intestine (Karabin et al., 2015).

During wort clarification, cooling, fermentation, maturation and beer stabilization, the polyphenol content is naturally significantly reduced by the precipitation of tannin-protein complexes due to cross interactions of components and pH reduction, and by the colloidal stabilization of beer directed to polyphenols (Siebert and Lynn, 2008; Aron and Shellhammer, 2010; Callemien and Collin, 2010; Steiner et al., 2010). High losses of prenylflavonoids are known to occur due to adsorption to the break and yeast cells (Stevens et al., 1999).

Especially the condensed, oxidized structures of catechins, proanthocyanidins, are considered to be haze-active polyphenols that form a colloidal haze particle with malt proteins and, in the case of colloidal beer stabilization, they are adsorbed to a polymeric sorbent acting as a model protein (Siebert and Lynn, 2008).

Changes in concentrations of simple flavanols and flavonols as well as glycosides of these substances and prenylflavonoids during beer maturation, primary beer filtration, filtration on membrane filters and colloidal stabilization of sorbents beer are not investigated.

The aim of our study was to obtain relevant information about the losses of polyphenols with a potential health benefit in the cold phase of brewing, fermentation and finishing operations.

2 Material and methods

To study the effect of the beer fermentation and maturation on polyphenolic substances (flavanoids, flavonoids and prenylflavonoids) in final beer, two 200 l pilot brews of pale lager were prepared (11% – original gravity brew/OG/ and 15% – high gravity brew /HGB/) using a double decoction mashing process. Investigation of the beer filtration and colloidal stabilization was carried out on another two brews of 11% pale lager. The commercial malt of the Bojos malting barley variety was used.

Hopping (CO_2 hop extract and Saaz pellets 90, 1:1) was in three doses, 30% (hop extract) at the beginning, 50% (hop extract+pellets) at 30 minutes and 20% (pellets) 10 minutes before the end of the boil. The low-pressure dynamic wort boiling took 70 minutes. The wort was clarified in a whirlpool, cooled with a plate cooler to a fermentation temperature of 10 °C and aerated to a dissolved oxygen concentration of 8 +/- 0.5 mg/L.

The wort main fermentation using the bottom type pitching yeast strain No RIBM95 was in cylindrical-conical tanks (CCT). The maximum temperature of the main fermentation was 12 °C ± 0.1 °C. When the difference between apparent and limit attenuation was about 10%, the beer was cooled to 5–6 °C within 24 hours and then transferred into lager tanks. Maturation was in a lager cellar at a temperature of 1–2 °C.

In the first two brews, an aliquot of 20 liters of beer from a 200-liter lager tank was filtered by a plate filter after 2 – 3 – 4 – 5 and 6 weeks. Filtration and bottling were under the protection of CO_2, 15% brew was adjusted to 11% with degassed and carbonized water.

In the second two batches, after 3 weeks of maturation the beers were filtered by a plate filter (depth filter plates of a mixture of cellulose, kieselguhr and perlite, type S10N with nominal retention of 0.8 μm, Hobra Školník, Czech Republic), 20 L aliquots of filtered beer were further filtered by a membrane filter unit with polyester sulfone membrane (PES module with 0.45 μm pores, Hobra Školník, Czech Republic) or have been treated with Vulcostabil 40C protein sorbent (Vulcascot, Austria) at a dose of 70 g/hl or treated with a Polyclar Super R polyphenol sorbent (Ashland, USA) at a dose of 50 g/hl. Filtration and bottling were under the protection of CO_2.

Basic beer analyzes and determination of total polyphenols were performed according to EBC-Analytica (2010), anthocyanogens were measured by the MEBAK method (Collection of Brewing Analysis Methods, 2013). Flavonoids were determined by liquid chromatography coupled to high resolution mass spectrometry (LC-HR/MS) on a Q-Exactive instrument (Thermo Fisher Scientific, Bremen, Germany).

The QuEChERS method (Anastassiades et al., 2003) was used to prepare beer samples. Flavonoids were extracted from a mixture of beer sample (10 ml) and acetonitrile (10 ml), after addition of a mixture of salts (4 g $MgSO_4$ and 1 g NaCl) followed by centrifugation (4500 rpm, 7 minutes). The acetonitrile layer containing the extracted flavonoids was separated. Next, one milliliter of this acetonitrile extract was first evaporated to dryness (Concentrator plus 5305, Eppendorf, Hamburg, Germany) and subsequently dissolved in one ml of methanol: water (1:1, v/v).

The LC-HR/MS assay was performed on a XSelect HSS T3 chromatography column (2.5 µm, 2.1 x 100 mm, Waters, Milford, MA, USA) with C18 reverse phase and the analytes were separated by gradient water elution (mobile phase A) and acetonitrile (mobile phase B) in both cases acidified by addition of 0.1% formic acid. The chromatographic separation was carried out at a column temperature of 40 °C and the column injection volume was 3 µl.

The data was recorded by a full-scan scanning mass spectrometer over a mass range of 120 to 900 m/z. The exact mass of the analyte of interest, calculated on the basis of the pseudomolecular ions summary formulas ([M-H] -), was extracted from the measured data with a mass accuracy of 5 ppm and further processed by TraceFinder v4.1 (Thermo Fisher Scientific, Waltham, MA, USA).

The content of individual flavonoids in beer samples was quantified using an external calibration curve constructed in the range of 10 to 200 µl/l for all analytes of interest. Quantified were: Flavanols (Catechin, Epicatechin, Catechin-O-glucoside, Epicatechin-O-glucoside), flavonols (Myricetin, Kvercetin, Kempferol, Rutin, Kvercetin-O-glucoside 1, Kvercetin-O-glucoside 2, Kaempferol-O- glucoside, Myricetin-O-glucoside, Multifidol-O-glucoside, Kvercetin-O-malonylglucoside) and prenylflavonoids (Isoxanthohumol, Xanthohumol, 8-prenylnaringenin, 6-prenylnaringenin).

3 Results and discussion

Fermentation and Maturation: The results of the analysis of worts, green beers and beers collected during the maturation period are given in Table 1 (11% brew – L1) and Table 2 (15% brew – L2). The wort and green beer data from the L2 brew are converted to the original wort extract of 11%. The results show a decrease in total polyphenols between wort, green beer and during four weeks of maturation. The concentration of total polyphenols and the trend of their changes were virtually identical in 11% (L1) and 15% (L2) brews. The lower polyphenols of high gravity brews reported in the literature (Basařová et al., 2010) were not observed.

Unlike total polyphenols, the concentration of flavan-3-ols (catechin and epicatechin) in worts and beers from the L2 brew was approximately 35% lower than the L1 brew. The value of total polyphenols includes all ferric ion reducing compounds, both phenolic acids and flavonoids are measured. The polyphenol compound profile of the L1 and L2 brews is likely to be different due to mash concentration differences during mashing and spent grain extraction at lautering. However, no final conclusions can be drawn from one experiment.

More than 50% of the catechin and epicatechin flavanols were significantly reduced after two to three weeks of maturation, while in the subsequent maturation period, between four and six weeks, the values remained virtually unchanged. In contrast, the level of glycosides, catechin-O-glucoside and epicatechin-O-glucoside in worts and beers was comparable for both batches, and did not change markedly during maturation after a drop of approximately 25% between wort and green beer. The flavanol glycosides are apparently more stable than the respective aglycones and, to a lesser extent, undergo physicochemical changes. Their amount in the experimental beers was about one quarter of the amount of free catechin or epicatechin.

The level of measured free flavonols, myricetin, quercetin and kaempferol in worts and beers, as well as the level of their glycosides, was slightly affected by fermentation and maturation. A slight decrease in flavonols depending on the maturation time was observed for quercetin and kaempferol-O-glucosides (10–20% decrease after 6 weeks of maturation).

A substantial decrease in the level of prenylflavonoids was observed after the first week of maturation (50% isoxanthonumol, 80% xanthohumol and 8- and 6-prenylnaringenin), but there were no significant decreases during the next maturation period. Isoxanthohumol is the dominant prenylflavonoid in beer, its losses in the maturation of beer were lower compared to the other substances measured in this group of polyphenols.

As follows from the above-mentioned analysis, the main flavonoid changes take place in the first two to three weeks of beer maturation. Extending the maturation time has no significant impact on the loss of flavonoid polyphenols, and shortening the maturation time of the beer would be reflected in the sensory profile of the final product.

Table 1 *Results of analysis of worts, green beers and beers for polyphenol content (ug/L) – 11% brew L1*

	L1_HW	L1_GB	L1_2W	L1_3W	L1_4W	L1_5W	L1_6W
Total Polyphenols (mg/l)	220	208	187	172	167	168	169
Flavanols (ug/l)							
Catechin	2505	2440	2327	1219	1093	1109	1216
Epicatechin	542	462	283	231	209	208	213
Catechin-O-glucoside	580	454	504	454	447	466	429
Epicatechin-O-glucoside	167	106	124	106	118	122	111
Flavonols (ug/l)							
Myricetin	80	82	77	77	77	77	77
Quercetin	37	54	54	31	27	32	26
Kaempferol	5	13	14	4	2	5	2
Rutin	110	119	128	142	118	135	118
Quercetin-O-glucoside 1	52	49	37	32	30	34	46
Quercetin-O-glucoside 2	397	369	369	397	310	389	327
Kaempferol-O-glucoside	213	189	190	203	171	196	189
Myricetin-O-glucoside	85	86	85	86	84	85	84
Multifidol-O-glucoside	104	96	103	106	91	101	97
Quercetin-O-malonylglucoside	18	101	92	102	84	98	87
Prenylflavonoids (ug/l)							
Isoxanthohumol	1033	1012	581	618	483	557	544
Xanthohumol	329	334	22	23	14	19	17
8-prenylnaringenin	116	112	19	20	13	17	16
6-prenylnaringenin	375	389	70	77	52	70	72

HW – hopped wort; GB – green beer; 2W – beer after 2 weeks of maturation

Filtration and stabilization: The results of the determination of polyphenol compounds in experimental beers from the second two batches are given in Table 3. After filtration of beer with depth filtration plates, a decrease of 10% of total polyphenols (TP) and 20% of anthocyanogens (ANT) was observed. After subsequent filtration of the beer with a PES polymer membrane filter, an additional 10% TP and 14% ANT were removed. It is thus likely that the depth filtration and PES membrane filter plates have an effect on the colloidal stability of the beer caused by the reduction of the polyphenol concentration. Stabilization of filtered beer with Vulcostabil 40C protein sorbent had only a negligible effect on the polyphenols.

The decrease between beer filtered with depth filter plates and beer subsequently stabilized with polyphenol sorbent was 12% of TP and 23% of ANT. The sorption of polyphenol substances by polymeric sorbents depends on the dose, contact time and the amount of sorbable polyphenols in the beer (Siebert and Lynn, 2008). Experimental beers filtered only by depth filter plates had very good haze stability and a true shelf life of 6 months, which explains the relatively low loss of polyphenols in beer treated with PVPP stabilizer.

Filtration of beer with depth filtration plates did not have a measurable effect on simple flavonoid polyphenols from the flavanol, flavonol and prenylflavonoid groups. From this knowledge it can be concluded that these plates only capture larger, oligomeric structures of polyphenols based on flavanols and proanthocyanidins. Stabilization of beer filtered with Vulcostabil 40C protein sorbent had only a negligible effect on flavonoid polyphenols. In contrast, the results of the analysis showed a marked decrease in the concentration of catechin (27%) and epicatechin (23%) after the filtration of the membrane by the membrane filter and an even higher decrease of these substances, 49% and 40% respectively, after stabilization by the sorbent of polyphenols.

Similarly, there was a significant decrease in some flavonols (quercerin, campferol) of about 25–40% and a decrease of their glucosides (quercetin-O-glucoside, kaempferol-O-glucoside) by 15–20% after filtration through the membrane filter as well as after stabilization by PVPP; the effect of membrane filtration was thus higher. In contrast, the level of rutin (quercetin-O-rutinoside) was not affected by either stabilization or membrane filtration.

Table 2 *Results of analysis of worts, green beers and beers for polyphenol content (ug/L) – 15% brew L2*

	L2_HW	L2_GB	L2_2W	L2_3W	L2_4W	L2_5W	L2_6W
Total Polyphenols (mg/l)	225	203	183	173	166	171	166
Flavanols (ug/l)							
Catechin	1899	1560	950	982	817	740	820
Epicatechin	331	245	231	184	142	128	140
Catechin-O-glucoside	517	381	417	377	402	460	416
Epicatechin-O-glucoside	133	125	123	99	97	92	108
Flavonols (ug/l)							
Myricetin	60	60	77	77	77	77	77
Quercetin	32	41	40	24	26	27	24
Kaempferol	6	11	9	1	2	2	1
Rutin	101	116	134	91	125	112	110
Quercetin-O-glucoside 1	46	41	33	30	37	45	44
Quercetin-O-glucoside 2	393	340	377	282	346	343	319
Kaempferol-O-glucoside	215	185	203	150	186	179	187
Myricetin-O-glucoside	64	64	85	83	84	84	83
Multifidol-O-glucoside	101	89	105	79	101	94	96
Quercetin-O-malonylglucoside	78	97	87	68	77	78	70
Prenylflavonoids (ug/l)							
Isoxanthohumol	936	764	557	443	509	477	453
Xanthohumol	337	228	30	17	21	14	14
8-prenylnaringenin	115	79	21	13	14	14	12
6-prenylnaringenin	385	339	94	57	69	57	63

HW – hopped wort; GB – green beer; 2W – beer after 2 weeks of maturation

Significant decreases in all investigated prenylflavonoids (23% isoxanthohumol, 45% xanthohumol) were observed after PVPP treatment, while even higher, 85% decreases were assessed after membrane filtration. The results of the experiment show that the content of beneficial flavonoid polyphenols in beer could be largely influenced not only by the treatment of beer with polyphenol sorbent but also by filtration by PES membranes. The extent of loss of polyphenol substances in membrane filtration will need to be further investigated.

4 Conclusion

The content of polyphenol substances with a potential impact on the quality of beer and the health benefit for the consumer is influenced by a number of factors, from raw materials, malt and hops. In our study, we focused on the "cold stage" of brewing production including final operation, filtration and colloidal stabilization of beer.

We found out fundamental changes of both total polyphenols and monomeric flavonoid phenolic compounds, namely the reduction of their final concentration in beer, occuring in the first two to three weeks of maturation. Glycosides of flavanols and flavonols undergo changes to a much lesser extent than free phenols. It is also likely that higher gravity worts contain lower amounts of malt flavonoid polyphenols.

Filtration of beer with depth filtration plates significantly reduced the number of total polyphenols and anthocyanogens, but it did not have a measurable effect on simple flavonoid polyphenols from the flavanol, flavonol and prenylflavonoid groups, so in the next work we will focus on the stabilizing effects of these plates.

Conversely, filtration by PES membrane filter reduced the number of flavonoids to a degree comparable to that of PVPP-based polyphenol sorbent. The content of beneficial flavonoid polyphenols in beer could be largely influenced not only by the treatment of beer with polyphenol sorbent, but also by filtration with membrane filters, which are used both for cold beer sterilization and for primary filtration as a replacement for kieselguhr filtration. This important finding should also be further explored.

Table 3 *Results of analysis of filtered and stabilized beers on polyphenol substances (ug/L)*

	NF	DP	DP + PES	DP + VULC	DP + PVPP
Total Polyphenols (mg/l)	256	230	207	220	203
Anthocyanogens (mg/l)	67	53	47	49	41
Flavanols (ug/l)					
Catechin	1423	1623	1292	1697	830
Epicatechin	532	492	485	511	455
Catechin-O-glucoside	218	254	216	259	152
Epicatechin-O-glucoside	95	95	92	99	90
Flavonols (ug/l)					
Myricetin	9	8	7	8	7
Quercetin	25	31	20	28	23
Kaempferol	14	21	13	20	16
Rutin	262	261	257	253	256
Quercetin-O-glucoside 1	38	37	28	36	31
Quercetin-O-glucoside 2	403	393	306	382	330
Kaempferol-O-glucoside	365	356	283	348	318
Myricetin-O-glucoside	12	12	11	12	11
Multifidol-O-glucoside	104	101	101	98	100
Quercetin-O-malonylglucoside	101	85	76	82	77
Prenylflavonoids (ug/l)					
Isoxanthohumol	820	785	123	728	605
Xanthohumol	97	57	10	42	31
8-prenylnaringenin	47	38	0	34	23
6-prenylnaringenin	180	151	22	131	98

NF – nonfiltered beer; DP – depth plate filtration; DP + PES – depth plate filtration + PES membrane; DP + VULC – depth plate filtration + Vulcostabil C40; DP + PVPP – depth plate filtration + Polyclar Super R

5 Acknowledgments:

The study was supported by the Technology Agency of the Czech Republic within the Competence Center, project TE02000177.

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