KVASNÝ PRŮMYSL

Kvasny prumysl (2024) 70: 846–854
https://doi.org/10.18832/kp2024.70.846

Study on the effect of malt and decoction mashing on polyphenols and antiradical power of wort

Alexandr Mikyška*, Marie Jurková

Research Institute of Brewing and Malting, Lípová 511/15, 120 00 Praha 2, Czech Republic

**corresponding author: mikyska@beerresearch.cz;*
ORCID: 0000-0001-7025-1436

Abstract

Mashing is one of the key operations in beer brewing. Together with polyphenolic compounds it can affect the quality and stability of beer. In our pilot brewing trials of pale lager (200 L), malts of three barley varieties were compared using either infusion or double decoction mashing. Total polyphenols, anthocyanogens and flavanoids were determined in sweet and hopped wort. Free phenolics were measured by HPLC coupled with coulometric detection. Antiradical power was determined by RC-DCPI, ARA-DPPH and ESR-T150 methods. In this way the influence of mashing method and barley variety on polyphenols and antiradical activity of sweet wort was demonstrated showing that the effect of mashing was stronger (ANOVA). The results showed significantly higher, i.e. by tens of per cent, levels of all polyphenols studied in both the decoction sweet wort and hopped wort. The decoction process resulted in higher levels of the antiradical power, ARA-DPPH (37–47%) and RC-DCPI (25–60%). A cluster analysis partitioned 28 free phenolic compounds in sweet wort primarily by variety and secondarily by mashing. The Malz malt showed a greater increase in polyphenols, free phenolic compounds and antiradical power in the decoction process compared to the Bojos and Sebastian malts. Decoction mashing introduces higher levels of phenolic antioxidants into the beer with the potential to improve the sensory stability of the beer and provide health benefits to the consumer. The different behaviour of malts and barley varieties needs to be better elucidated in further research.

Keywords: mashing; malt; barley variety; polyphenols; free phenolic compounds; antiradical activity

1 Introduction

Polyphenolic substances influence beer quality, colloidal (Wannemacher et al., 2018; Steiner et al., 2010) and most probably also sensory stability (Mikyška et al., 2022; Guido et al., 2007). They are a very diverse group of substances whose individual components are characterised by different properties in terms of antioxidant capacity, haze forming properties and thus influence on the sensory stability of beer, as well as affinity for colloidal haze formation. Some simple and more complex polyphenols, either alone or due to their oxidation products, are sensory active and influence the bitterness and astringency of beer (Callemien et al., 2005).

Beer polyphenols originate from malt and hops, whose components differ significantly in chemical structure, and therefore they have different properties in terms of antiradical and metal chelating functions (Radonjic et al., 2020; Wannenmacher et al., 2018). Both malt and hops contain phenolic acids (e.g., ferulic acid, gallic acid), monomeric and oligomeric flavonoids, in particular flavanol monomers (e.g., catechin), oligomeric proanthocyanidins, and flavonols (e.g., quercetin). The composition of the polyphenolic substances of hops and malt is particularly different, e.g., malt has a higher content of phenolic carboxylic acids than hops, which, conversely, contain more flavonols and their glycosides (Wannenmacher et al., 2018). Flavonoids, especially flavonols (quercetin, myricetin, kaempferol, and their glycosides such as rutin (quercetin-O-rutinoside) are con-

Research Institute of Brewing and Malting
Published online: 15 February 2024

sidered to be very effective antioxidants, scavenging free radicals, inhibiting oxidative enzymes and chelating trace elements (metal divalent cations) involved in free radical pathways (Nowak et al., 2014).

The content of phenolic substances in beer depends on the malt used (Wannenmacher et al., 2018). Malt polyphenols are situated in the cell walls of the coating layers and the endosperm of the grain, bound to proteins and non-starch polysaccharides (Holtekjölen et al., 2006), therefore, their content in wort could be affected by both the malt modification and the mashing. The antiradical power of malt, correlating with the content of polyphenols in malt, is a major contribution of malt to the sensory stability of beer (Guido et al., 2007). The content of polyphenols in beer also depends on the hop products and hopping technology (Mikyška et al., 2022; Wannenmacher et al., 2019).

The content and composition of polyphenolic substances in beer depends on a number of factors, both the origin and composition of the raw materials and the way the technological process is carried out in the malt house and brewery. The production of wort is one of the key operations in brewing and this step lays the foundation for the formation of the full range of sensory active compounds in beer, i.e. a colour, foam, body and other important characteristics of the beverage.

The aim of mashing is to convert the desirable extractable malt components into a soluble form with the help of malt enzymes at a rate and composition appropriate for the beer to be produced (Basařová et al., 2017). It is undeniable that the analytical and sensory image of beer is determined by a combination of raw material and technological factors. This is true for different beer styles as well as for variability within a single style. There are two basic methods of producing sweet wort. In the time and energy saving infusion process, the entire mash is treated with heating steps that match the enzyme activity. In the decoction process, part of the mash is isolated and heated in a separate kettle. The boiled part of the mash is returned to the rest of the mash. Both processes have a number of variations depending on the malt and beer style that is being processed (Basařová et al., 2017).

In our previous study (Mikyška et al., 2023) we showed a significant effect of mashing intensity on total polyphenols and flavonoids (proanthocyanidins, flavonoids and prenylflavonoids), with decoction methods resulting in higher levels in both the wort and final beer. The aim of this work was (i) to investigate the effect of malt (a barley variety) on the changes in polyphenolic compounds as a function of mashing intensity and (ii) to determine the effect of mashing on flavonoid and non-flavonoid polyphenols, phenolic acids.

2 Materials and methods

2.1 Wort production

Pilot-scale brewing trials (200 L), with commercial pilsner malt of the three barley varieties, were performed in duplicate in the pilot brewery of the Research Institute of Brewing and Malting (RIBM). The malt grist was prepared using a 2-roller mill. The ratio of the malt grist to water in mashing-in was 1:3.7. The mashing of all malt 12% brews was carried out by infusion (INF) and double-decoction (DEC) protocols (Figure 1).

The mash solids were separated from the sweet wort using a lauter tun, keeping the total volume of the sweet wort constant. Hopping doses were 50% (CO_2 extract) at the onset, 35% (Saaz pellets 90) after 30 min and 15% (Saaz pellets 90) 10 min before the end of the 70-min wort boiling process. After hot trub separation in a whirlpool, the wort was cooled to a fermentation temperature of 10 °C. Samples of sweet wort and cold wort were analysed.

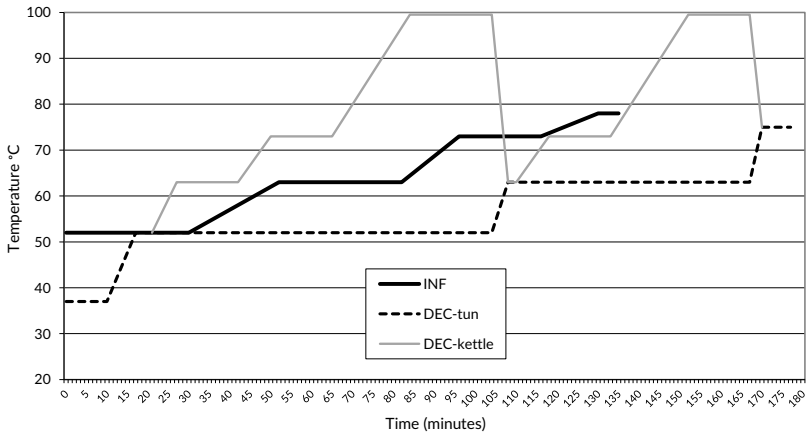

Figure 1 *Mashing diagrams of infusion (INF) and decoction (DEC) procedures*

2.2 Analysis

The analysis of malts and hop products as well as of total polyphenols (TP) and flavonoids (FLA) was carried out according to the EBC Analytics (Analytica-EBC, 2010). The reducing capacity (2,6-dichlorophenol indophenol; RC-DCPI) and anthocyanogens (ANT) was determined

using the MEBAK analytical methods (MEBAK, 2013). The antiradical activity, using the stable free radical DPPH (1,1-dipyridyl-2-picryl hydrazyl; ARA-DPPH), was determined by a protocol developed previously (Mikyška et al., 2006). The endogenous antiradical activity, T 150 value of sweet wort, wort and hops was determined according to the procedure published by Ushida et al. (1996).

Free phenolics comprising 28 compounds were determined using HPLC coupled with coulometric detection according to the procedure previously described by Jurková et al. (2010). The quantified compounds included flavonoids: flavanols (catechin, epicatechin), flavonols (myricetin, quercetin, rutin), flavanone (naringin) and flavon (apigenin); free phenolic acids: hydroxycinnamic acids (ferulic, synaptic, p-coumaric, chlorogenic and caffeic acid), hydroxybenzoic acids (p-hydroxybenzoic, gallic, protocatechuic, gentisic, vanillic and syringic); 4-hydroxyphenylacetic acid; hydroxycoumarins (4-hydroxycoumarin, umbelliferon, esculin and scopoletin). The results are given in mg/L of wort.

Analytical data were processed by a two-factor analysis of variance (ANOVA) and a cluster analysis.

3 Results and discussion

Commercial pale malts of three spring malting barley varieties grown in the Czech Republic were used for the experiments. The malts were of comparable malting quality, with parameters meeting the requirements for malt according to the PGI Czech Beer (European Commission, 2008), i.e. a lower proteolytic and saccharolytic modification. Therefore, a mash-in temperature of 52 °C was used for the infusion process. Bojos and Malz malts had the RE45 and Kolbach index lower than Sebastian malt, Bojos malt had a limit attenuation lower than the other two malts. The content of total polyphenols and anthocyanogens in the malts (laboratory wort) decreased in the order Bojos, Malz and Sebastian (Table 1).

3.1 Sweet wort

The extract concentrations of the experimental wort ranged from 10.6 to 11.9%, so the analytical results were recalculated to an extract of 12% for a comparison. As can be seen from the average values for the malts and mashing technology used (Table 2), the wort from all three malts produced by the decoction process showed significantly higher concentrations of all polyphenolic compounds evaluated by the group methods. The rate of increase slightly varied for the malts used, with the highest increase in total polyphenols found for the Malz malt (53%), and the difference for the Bojos and Sebastian varieties being virtually identical at 28 and 30% respectively.

Total polyphenols include both flavonoids and phenolic acids and their determination is based on the reaction of polyphenols with ferric ions (ferric ammonium citrate) in an alkaline medium forming a red colour complex, which is determined photometrically. It should be borne in mind that the molar extinction coefficients of the polyphenolic compounds are different and that other reducing agents capable of reducing ferric ions to ferrous ions may interfere.

Table 1 *Analytical parameters of malts*

Barley Variety		Malz	Bojos	Sebastian
Colour	EBC	2.8	2.8	4.2
Colour after boiling	EBC	5.8	4.8	5.7
pH		6.16	6.14	6.11
Extract	%	82.9	83.9	83.5
Extract difference DLFU	%	0.9	0.7	1.0
Rel. extract 45 °C	%	30.6	28.4	35.0
Diastatic power	WK	300	297	305
Attenuation	%	79.7	75.1	80.1
Viscosity	mPa.s	1.50	1.45	1.48
Protein	%	9.9	9.4	8.9
Soluble nitrogen	mg/100 mL	67.5	64.1	64.7
Kolbach index	%	37.9	38.2	40.7
Friability	%	88.7	92.5	85.8
Homogenity	%	71.0	95.4	71.0
Modification	%	93.0	82.7	90.4
Total polyphenols	mg/L	70	68	74
Anthocyanogens	mg/L	23.4	21.3	24.1

Table 2 *Polyphenols content and antiradical power of sweet worts*

Barley variety		Malz				Bojos				Sebastian			
Mashing protocol		INF		DEC		INF		DEC		INF		DEC	
		R	SD	R	SD	R	SD	R	SD	R	SD	R	SD
Colour	EBC	9.13	0.49	10.20	0.02	6.83	0.09	8.03	0.19	8.00	0.11	8.85	0.27
pH		5.91	0.02	5.82	0.05	5.75	0.15	5.85	0.05	6.03	0.02	6.08	0.00
Total polyphenols	mg/L	95.1	1.1	145.3	2.9	102.1	7.8	130.3	1.4	110.3	1.4	143.6	1.7
Anthocyanogens	mg/L	25.1	1.1	37.2	2.0	22.4	0.8	35.9	1.5	26.1	0.7	38.5	0.6
Flavanoids	mg/L	12.9	0.3	21.3	0.0	9.9	0.0	15.5	0.4	15.2	1.0	21.7	1.3
RC-DCPI	% rel	19.4	1.1	38.8	2.8	33.4	0.2	41.8	0.7	34.2	2.0	43.1	0.4
ARA-DPPH	% rel	41.9	0.4	57.6	0.1	34.4	1.2	50.6	0.6	40.2	2.5	57.9	1.6
ESR-T150		3.51	0.49	7.38	0.59	4.26	0.30	5.61	0.17	3.53	0.47	5.00	0.49
RC-DCPI – reducing capacity, ARA-DPPH – antiradical activity, ESR-T150 – endogenous antiradical activity													

Barley contains a several groups of phenolic compounds, mainly phenolic acids (free and bound). Their total content varies from 60.4 to 134.6 mg/100 g of barley flour, and the flavanol content varies from 32.5 to 52.7 mg/100 g of barley flour (Holtekjölen et al., 2006). The increase in total polyphenols during mashing is caused by the release and solubilisation of phenolics from malt due to the action of hydrolytic enzymes and water extraction (Zhao and Zhao, 2012; Vanbeneden et al., 2008; Pascoe et al., 2003).

It has been reported that the total polyphenols in the mash gradually increase with a prolonged mashing time and an increased temperature until the mashing temperature reaches 78 °C (Zhao and Zhao, 2012). Oxidation, degradation and formation of precipitated protein-polyphenol complexes and polyphenol polymers could partly explain the decrease in TPC at high mashing temperatures above 78 °C (Aron and Shellhammer, 2010). The results of these experiments confirmed our previous finding (Mikyška et al., 2023) that a longer decoction mashing time contributes to the release of polyphenolic substances, while mash boiling does not have a negative effect on the total polyphenol content in sweet wort.

The anthocyanogen content of decoction sweet wort increased by about half (48 to 60%) for all three varieties. For Bojos and Sebastian malts, both the absolute and relative anthocyanogen content of decoction sweet wort was higher than that of infusion sweet wort, i.e. a lower polymerisation index (ratio of total polyphenols to anthocyanogens). Anthocyanogens are a group of leucocyanidins (standard delphinidin chloride) which react in acidic environments to form red oxonium salts, determined photometrically. These substances have been attributed to a significant influence on the formation of three-protein haze and deterioration of colloidal stability of beer (Wannenmacher et al. 2018; Basařová et al. 2017).

The difference in the flavanoid content between decoction and infusion sweet wort was highest for Malz malt (65%) and lowest for Sebastian malt (42%). Flavanoids include monomeric catechins (flavan-3-ols) and oligomeric proanthocyanidins (chromogen p-dimethylcinnamaldehyde, catechin standard).

For all groups of polyphenolic compounds, a very strong dependence (ANOVA) on the mashing procedure used was found; the effect of malt was detected at a probability level higher than 0.05 only for flavanoids (Table 3).

The results of the analysis of free phenolic compounds in the wort (Table 4) showed both the varietal specificity of the composition of these compounds and, similarly to the polyphenolic compounds determined by group methods, the influence of the mashing technology on the content of individual free phenolic compounds in the wort. Regarding the effect of mashing, the results are in agreement with previously published findings (Jurková et al., 2012). For the sake of clarity, the substances are sorted according to affinity, i.e. chemical structure. For example, higher levels of the flavonoids rutin, biochanin A and ferulic acid were found in the sweet wort from the variety Malz, while higher levels of the flavonoids myricetin and quercetin were found in the sweet wort from the variety Sebastian.

The increase in the sum of compounds was highest in decoction sweet wort made with Malz malt (by 48%), mainly due to an increase in the concentration of flavanoids (by 64%), while the increase in the sum of markers was comparable for Bojos (by 39%) and Sebastian (by 37%) malts. The content of hydroxycinnamic acids in decoction sweet wort was higher by 26 to 34%. The higher content of hydroxycinnamic acids in the double decoction wort was probably due to the lower temperature of the mash-in (37 °C versus 52 °C). The enzymes releasing these substances are highly thermolabile (Basařová et al., 2017).

A data analysis showed a greater dependence on the variety than on the mashing procedure for flavanoids and hydroxybenzoic acids, while the opposite was true for hydroxycoumarins (Table 3). The cluster analysis of 26 free phenolic compounds (Figure 2) separated the variants by a barley variety in the first level of the hierarchy and by a mashing method in the second level.

The reducing properties of beer and its production intermediates depend on the production process and raw materials (Basařová et al., 2017). Both malt (Guido et al., 2007) and hops (Mikyška et al., 2022; Mertens et al., 2020) are involved.

The RC-DCPI reducing capacity of the decoction wort was higher, a significant difference (76%) was found for Malz malt, the increase for Bojos and Sebastian was 25 and 26% respectively. The barley variety also had a significant effect (P=0.001). The RC-DCPI evaluates to a greater extent the fast reductones, sugar reductones and melanoidinins, reaction products of Maillard reactions of amino acids with carbohydrates (Kaneda et al., 1999), whose formation is promoted by the higher heat load and longer process time of the double decoction mashing process (Basařová et al., 2017). As expected, the decoction wort was found to have a higher colour, but the malt used had a stronger influence on the colour (Table 3).

Table 3 *Effect of mashing and malt on sweet wort composition (ANOVA)*

	F	P value	F crit		F	P value	F crit
	TP				RC-DCPI		
Mashing	160.43	1E-05	5.987		98.54	6E-05	5.987
Malt	4.52	0.063	5.143		24.03	0.001	5.143
	ANT				ARA-DPPH		
Mashing	161.57	1E-05	5.987		229.38	5E-06	5.987
Malt	3.40	0.103	5.143		18.04	0.003	5.143
	FLA				ESR-T150		
Mashing	143.40	2E-05	5.987		38.29	8E-04	5.987
Malt	37.21	4E-04	5.143		3.61	0.093	5.143
	pH				Colour		
Mashing	0.11	0.754	5.987		26.64	0.002	5.987
Malt	7.24	0.025	5.143		40.88	3E-04	5.143
	Flavonoids				Hydroxycinnamic acids		
Mashing	162.06	1E-05	5.987		22.56	0.003	5.987
Malt	38.96	4E-04	5.143		28.81	8E-04	5.143
	Hydroxybenzoic acids				Hydroxycoumarins		
Mashing	46.93	5E-04	5.987		9.20	0.023	5.987
Malt	0.65	0.555	5.143		84.32	4E-05	5.143

TP – total polyphenols, ANT – anthocyanogens; FLA – flavanoids, RC-DCPI – reducing capacity, ARA-DPPH – antiradical activity, ESR-T150 – endogenous antiradical activity

The antiradical activity of ARA-DPPH increased by 37 to 47% in decoction brews of all varieties compared to the infusion procedure at comparable rates. ARA-DPPH correlated more strongly with total polyphenols, anthocyanogens and flavanoids (r=0.921, 0.0955, 0.905 respectively) than with RC-DCPI (r=0.810, 0.745, 0.575 respectively). The results are consistent with the finding of a close relationship between antiradical activity and the concentration of polyphenols in wort (Zhao and Zhao, 2012; Szwajgier, 2009; Guido et al., 2007). The free radi-

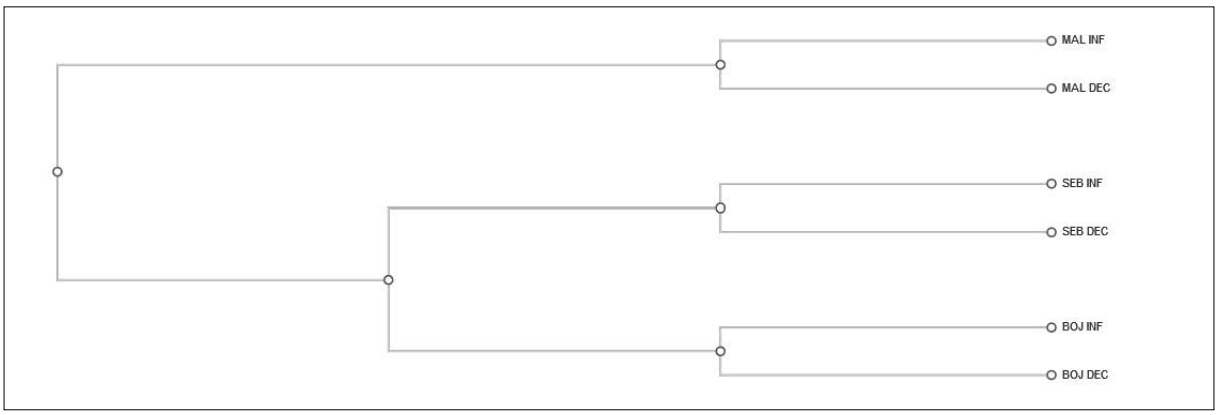

Figure 2 *Cluster analysis of free phenolic compounds in sweet wort*
MAL- Malz, BOJ – Bojos, SEB – Sebastian, INF – infusion, DEC – decoction mashing

Table 4 *Free phenolic compounds in sweet wort (mg/L)*

Barley variety	Malz				Bojos				Sebastian			
Mashing protocol	INF		DEC		INF		DEC		INF		DEC	
	R	SD	R	SD	R	SD	R	SD	R	SD	R	SD
Catechin	1.89	0.22	2.77	0.21	1.91	0.06	2.81	0.13	1.67	0.14	2.32	0.02
Epicatechin	0.14	0.03	0.23	0.04	0.66	0.01	0.81	0.09	0.20	0.00	0.24	0.00
Rutin	0.64	0.24	1.75	0.20	0.13	0.00	0.30	0.01	0.22	0.02	0.44	0.13
Myricetin	0.00	0.00	0.00	0.00	0.17	0.02	0.19	0.00	0.59	0.04	0.79	0.00
Quercetin	0.08	0.03	0.06	0.02	0.06	0.00	0.07	0.00	0.38	0.05	0.52	0.04
Naringin	1.81	0.10	2.53	0.23	1.14	0.01	1.35	0.15	0.75	0.03	1.37	0.23
Apigenin	0.22	0.11	0.17	0.00	0.07	0.02	0.08	0.00	0.24	0.01	0.32	0.01
Daidzein	0.03	0.01	0.02	0.01	0.06	0.00	0.07	0.00	0.44	0.00	0.59	0.04
Genistein	0.02	0.01	0.03	0.01	0.03	0.00	0.03	0.00	0.20	0.02	0.30	0.06
Formononethin	0.03	0.01	0.96	0.18	0.33	0.05	0.53	0.03	0.25	0.06	0.45	0.05
Biochanin A	0.84	0.82	1.56	0.35	0.11	0.01	0.14	0.06	0.09	0.04	0.14	0.03
Flavonoids	**5.69**	**0.36**	**10.08**	**0.37**	**4.68**	**0.02**	**6.38**	**0.29**	**5.03**	**0.31**	**7.46**	**0.01**
Ferulic acid	5.22	0.59	6.01	0.30	1.89	0.04	2.17	0.10	2.57	0.11	3.56	0.18
Sinapic acid	0.39	0.02	0.82	0.33	0.39	0.00	0.49	0.01	0.21	0.11	0.26	0.13
Coumaric acid	0.18	0.06	0.46	0.12	0.96	0.00	1.23	0.01	0.98	0.47	1.23	0.60
Chlorogenic acid	0.13	0.03	0.16	0.02	0.30	0.04	0.61	0.09	0.18	0.02	0.22	0.03
Caffeic acid	0.12	0.01	0.22	0.01	0.41	0.00	0.47	0.02	0.11	0.04	0.14	0.05
Hydroxycinnamic acids	**6.04**	**0.62**	**7.68**	**0.12**	**3.94**	**0.00**	**4.98**	**0.23**	**4.05**	**0.44**	**5.41**	**0.28**
p–Hydroxybenzoic acid	1.00	0.12	1.92	0.31	0.00	0.00	1.34	0.13	1.03	0.20	1.39	0.37
Gallic acid	0.02	0.00	0.03	0.00	0.19	0.04	0.28	0.06	0.41	0.02	0.57	0.01
Protocatechuic acid	0.09	0.00	0.14	0.01	0.28	0.00	0.34	0.01	0.12	0.01	0.15	0.01
Gentisic acid	0.03	0.01	0.04	0.02	0.00	0.00	0.35	0.00	0.04	0.02	0.05	0.03
Vanillic acid	0.36	0.03	0.42	0.02	0.51	0.03	0.59	0.01	0.41	0.03	0.56	0.00
Syringic acid	0.29	0.04	0.32	0.01	0.27	0.00	0.29	0.01	0.10	0.01	0.12	0.01
Hydroxybenzoic acids	**1.79**	**0.05**	**2.88**	**0.34**	**1.25**	**0.01**	**3.19**	**0.18**	**2.11**	**0.18**	**2.84**	**0.35**
4–Hydroxycoumarin	0.10	0.02	0.11	0.05	0.07	0.00	0.08	0.00	0.24	0.04	0.34	0.10
Umbelliferon	0.23	0.02	0.52	0.23	0.56	0.40	0.30	0.00	1.20	0.06	1.49	0.06
Esculin	0.21	0.20	0.72	0.27	0.99	0.06	1.09	0.12	1.64	0.02	2.04	0.05
Scopoletin	0.12	0.07	0.08	0.04	0.03	0.01	0.06	0.01	0.02	0.01	0.02	0.01
Hydroxycoumarins	**0.63**	**0.09**	**1.43**	**0.02**	**1.65**	**0.45**	**1.52**	**0.14**	**3.09**	**0.00**	**3.89**	**0.10**
Vanillin	0.32	0.03	0.31	0.04	0.65	0.04	0.83	0.05	0.35	0.01	0.45	0.03
4–Hydroxyphenylacetic acid	0.17	0.00	0.18	0.01	0.30	0.05	0.39	0.01	0.35	0.01	0.44	0.01
Total free phenolics	**14.61**	**0.15**	**22.55**	**0.66**	**12.46**	**0.44**	**17.28**	**0.27**	**14.97**	**0.95**	**20.48**	**0.76**

cal DPPH reacts mainly with the slow reductones and the polyphenols (Kaneda et al., 1999).

Higher values of ESR-T150 antiradical activity were found for decoction wort, with a non-negligible deterioration compared to infusion wort. The highest difference (53%) was observed for the Malz malt wort, while a lower deterioration was observed for the Bojos (26%) and Sebastian (30%) malts. The effect of malt was inconclusive (Table 3). This method is used to evaluate the activity of hydroxyl radicals, which are involved in undesirable oxidative changes of extract components in the chain of radical reactions (Ushida et al., 1996; Mertens et al., 2020).

The Bojos and Sebastian malts were comparable in terms of differences in the polyphenol content and antioxidant activity between the infusion and decoction mashing treatments. A higher degree of dependence on the mashing technique was observed for the Malz malt. Bojos is currently the most cultivated variety in the Czech Republic, while Malz and Sebastian are older varieties. The content of polyphenolic compounds in barley and their antiradical properties depend on both a variety and location (Mikyška et al., 2019; Mareček et al., 2017). Total polyphenols in laboratory worts range from about 55 to 85 mg/L in currently domestically grown malting barley varieties (Psota et al., 2022). The variance of malting parameters is considerable across the world's genetic resources (Zavrelova et al., 2021).

3.2 Hopped wort

Hops are a source of polyphenolic substances with a different profile compared to malt, with a predominant proportion of flavonoid polyphenols (Mikyška et al., 2019; Wannenmacher et al., 2018). During boiling of wort with hops, hop polyphenols are released and reactions of malt and hop polyphenols with other components of the wort extract occur (Wannenmacher et al., 2018; Basařová et al., 2017). In addition to the aforementioned, also malt polyphenols and hop polyphenols are found in the wort.

Table 5 *Results of hop raw material analyses*

		Hop products*	
		Extract	Pellets
Alpha-acids	%	50.3	3.9
Beta-acids	%	28.0	4.7
Total polyphenols	mg/L	7.2	276
Anthocyanogens	mg/L	8.5	123
Flavanoids	mg/L	0.0	27.5
Flavonoids (HPLC)	mg/L	0.40	24.39
Phenolic acids (HPLC)	mg/L	0.19	0.84
RC-DCPI	% rel	3	19
ARA-DPPH	% rel	2	83
ESR-T150		0.10	1.98

RC-DCPI – reducing capacity, ARA-DPPH – antiradical activity, ESR-T150 – endogenous antiradical activity
* polyphenols and antioxidant activity of boiling water extract 5 g/L

The experimental brews were hopped with 50% Saaz pellets with a high content of polyphenolic substances and reducing power (Mikyška and Jurková, 2019) and 50% hop CO_2 extract with a practically negligible content of polyphenols (Table 5). Even in the hopped wort, the infusion and decoction mashing procedure was clearly different. The content of total polyphenols and anthocyanogens in the wort was on average about one third higher in the decoction compared to the infusion (Table 6). For all polyphenol groups studied, both the effect of mashing and the effect of malt were evident, but the dependence of the polyphenol concentration on malt was weaker (Table 7).

The content of free phenolic compounds in the wort of the decoction brews was 38–42% higher than in the infusion brews. The contribution of hops was reflected in an increase in flavanoid content in all brews, while phenolic acid levels decreased (Figure 3).

Both the reducing power of RC-DCPI and the antiradical activity of ARA-DPPH increased significantly in hopped wort compared to sweet wort, and the differ-

Table 6 *Polyphenols content and antiradical power of hopped worts*

Barley variety		Malz				Bojos				Sebastian			
Mashing protocol		INF		DEC		INF		DEC		INF		DEC	
		R	SD	R	SD	R	SD	R	SD	R	SD	R	SD
Total polyphenols	mg/L	153.5	1.5	211.2	3.2	123.4	2.4	181.1	2.7	147.2	0.8	189.2	2.8
Anthocyanogens	mg/L	36.9	0.5	50.7	1.2	33.9	0.5	44.9	0.9	37.15	1.05	50	2
Flavanoids	mg/L	16.7	0.1	27.5	0.9	12.5	0.4	15.2	0.4	16.9	1.2	23.2	0.2
RC-DCPI	% rel.	52.1	1.1	69.4	1.0	58.0	0.6	66.4	1.4	59.2	1.2	69.0	1.6
ARA-DPPH	% rel.	57.4	0.8	72.0	0.8	46.8	0.4	59.3	1.9	58.2	0.6	73.0	0.8
ESR-T150		1.89	0.10	3.21	0.21	1.91	0.13	2.61	0.09	2.14	0.16	3.43	0.13

Table 7 *Effect of mashing and malt on hopped wort composition (ANOVA)*

	F	P value	F crit		F	P value	F crit
	TP				RC-DCPI		
Mashing	728.24	2E-07	5.987		147.74	2E-05	5.987
Malt	79.99	5E-05	5.143		3.97	0.08	5.143
	ANT				ARA-DPPH		
Mashing	180.52	1E-05	5.987		294.65	3E-06	5.987
Malt	9.38	0.014	5.143		98.28	3E-05	5.143
	FLA				ESR-T150		
Mashing	157.45	2E-05	5.987		90.10	8E-05	5.987
Malt	88.31	4E-05	5.143		6.82	0.028	5.143

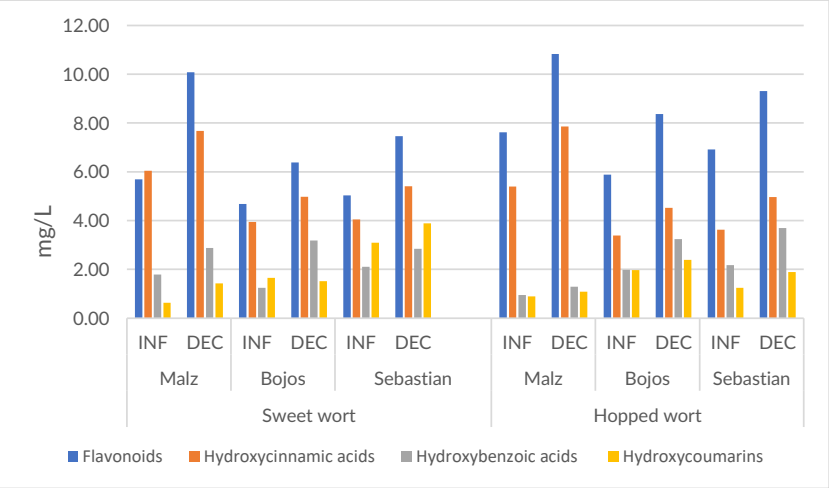

Figure 3 *Comparison of the concentration of free phenolic compounds in sweet wort and the hopped wort*

ences between decoction and infusion wort decreased. However, the RC-DCPI and ARA-DPPH of the decoction hopped wort were on average 21% higher than those of the infusion hopped wort. There was a clear relationship with ARA-DPPH for malt (Table 7). The antiradical activity of ESR-T150 was significantly improved compared to wort; hop polyphenols and bitter acids have antiradical properties, the ability to quench free radicals and also to chelate transition metal ions, catalysts of radical reactions (Mikyška et al., 2022; Mertens et al., 2020). For the ESR-T150 value of the hopped wort, both the relationship with the mashing process and the relationship with the malt were conclusive.

The results of the experiments clearly confirmed that the sweet wort and subsequently the hopped wort produced by the decoction mashing process had significantly higher contents of polyphenolic compounds (by 20 to 40%) and free phenolic compounds (by 25 to 35%) and better antioxidant properties, as assessed by the RC-DCPI (by 20 to 50%) and ARA-DPPH (by 27 to 32%) methods, compared to the infusion process. The degree of the increase in the polyphenol content, free phenolic compounds and antiradical properties between the infusion and decoction processes depended on the barley variety, with greater differences found for Malz malt than for Bojos and Sebastian malt.

4 Conclusion

Experimental brews aimed to elucidate the influence of decoction mashing and barley variety on the content and profile of polyphenols and free phenolic compounds in sweet wort showed significantly higher, by several tens of per cent, levels of all polyphenols studied in the decoction sweet wort as well as in the hopped wort. The decoction process resulted in a higher antioxidant capacity of the sweet wort, antiradical activity of ARA-DPPH and a reducing capacity of RC-DCPI. The barley variety also appears to be a significant factor in relation to mashing intensity, with Malz malt showing a greater increase in polyphenols, free phenolic compounds and antiradical power in the decoction process than the other two barley varieties tested. Decoction mashing introduces higher levels of phenolic antioxidants into the beer with the potential to improve the sensory stability of the beer and provide health benefits to the consumer. The different behaviour of the malts and barley varieties needs to be better elucidated in further research.

5 Acknowledgement

The study was supported by the Ministry of Agriculture of the Czech Republic within the Institutional Support MZE-RO1923.

6 References

Analytica EBC (2010). European Brewery Convention. Carl-Hans Verlag, Nürenberg. ISBN 978-3-418-00759-5

Aron, P.M., Shellhammer, T.H. (2010). A discussion of polyphenols in beer physical and flavour stability. Journal of the Institute of Brewing, 116, 369–380. https://doi.org/10.1002/j.2050-0416.2010.tb00788.x

Basařová, G., Šavel, J., Basař, P., Lejsek, T. (2017). The comprehensive guide to brewing. Fachverlag Hans Carl, Nürenberg. ISBN 978-3418008424

Callemien, D., Bennani, M., Counet, C., Collin, S. (2005) Which polyphenols are involved in aged beer astringency? Assessment by HPLC and time-intensity method. Proceedings of the 30th Congress of the European Brewery Convention, Prague, Fachverlag Hans Carl, Nurnberg, CD-ROM, 809–814.

European Commission (2008) [online]. https://eur-lex.europa.eu/legal-content/CS/TXT/?uri=OJ:C:2008:016:TOC [accessed 2024-01-25].

Guido, L.F., Curto, A.F., Boivin, P., Benismail, N., Goncalves, C.R., Barros, A.A. (2007). Correlation of Malt Quality Parameters and Beer Flavor Stability: Multivariate Analysis. Journal of Agricultural and Food Chemistry, 55, 728–733. https://doi.org/10.1021/jf0623079

Holtekjölen, A.K., Kinitz, C., Knutsen, S.H. (2006). Flavanol and bound phenolic acid contents in different barley varieties. Journal of Agricultural and Food Chemistry, 54, 2253–2260. https://doi.org/10.1021/jf052394p

Jurková, M., Horák, T., Hašková, D., Čulík, J., Čejka P., Kellner, V. (2012). Control of antioxidant beer activity by the mashing process. Journal of the Institute of Brewing, 118, 230–235. https://doi.org/10.1002/jib.35

Jurková, M., Kellner, V., Čulík, J., Horák, T., Čejka, P., Karásek, P. (2010). Analysis of polyphenols in brewing raw materials by PSE (Pressurized Solvent Extraction) – and by HPLC method with CoulArray detection. Kvasny prumysl, 56(1), 18–23. https://doi.org/10.18832/kp2010003

Kaneda, H., Kobayashi, N., Furusho, S., Sahara, H., Koshino, S. (1999). Reducing activity and flavor stability of beer. Techechnical Quarterly MBAA, 32, 90–94.

Mareček, V., Mikyška, A., Hampel, D., Čejka, P., Neuwirthová, L., Malachová, A., Cerkal, R. (2017). ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. Journal of Cereal Science, 72, 40–45. https://doi.org/10.1016/j.jcs.2016.11.004

MEBAK (2013). Collection of Brewing Analysis Methods of the Mitteleuropäische Brautechnische Analysenkommission. Wort, Beer, Beer-based beverages, Fachverlag Hans Carl, Nürenberg. ISBN 978-3-9805814-7-9

Mertens, T., Kunz, T., Methner, F.-J. (2020). Assessment of chelators in wort and beer model solutions. Brewing Science, 73(5-6), 58–67. https://doi.org/10.23763/BrSc20-01mertens

Mikyška, A., Dušek, M., Čejka, P. (2019). Influence of barley variety and growing locality on the profile of flavonoid polyphenols in malt. Kvasny prumysl, 65(5), 149–157. https://doi.org/10.18832/kp2019.65.149

Mikyška, A., Jurková, M. (2019). Varietal specificity of polyphenols, free phenolics and antioxidant potential in hops. Kvasny prumysl, 65(6), 178–185. https://doi.org/10.18832/kp2019.65.178

Mikyška, A., Jurková, M., Horák, T., Slabý, M. (2022). Study of the influence of hop polyphenols on the sensory stability of lager beer. European Food Research Technology, 248, 533–542. https://doi.org/10.1007/s00217-021-03900-0

Mikyška, A., Krofta, K., Hašková, D. (2006). Evaluation of antioxidant properties of hop and hop products. Kvasny prumysl, 52, 214–218. https://doi.org/10.18832/kp2006020

Mikyška, A., Dušek, M., Slabý, M. (2023). Effects of the mashing process on polyphenols and antiradical activity of beer. European Food Research Technology, 249, 71–80. https://doi.org/10.1007/s00217-022-04133-5

Nowak, R., Olech, M., Nowacka, N. (2014). Plant polyphenols as chemopreventive agents. In: Watson, R., Preedy, V., Zibadi, S., (eds.). Polyphenols in human health and disease. Elsevier, San Diego, 1289–1307. https://doi.org/10.1016/B978-0-12-398456-2.00086-4

Pascoe, H.M., Ames, J.M., Sachin, C. (2003). Critical stages of the brewing process for changes in antioxidant activity and levels of phenolic compounds in ale. Journal of the American Society of Brewing Chemists, 61(4), 203–209. https://doi.org/10.1094/ASBCJ-61-0203

Psota, V., Dvořáčková, O., Musilová, M. (2022). Barley varieties registered in the Czech Republic after the harvest of 2021. Kvasny Prumysl, 68(2), 590–601. https://doi.org/ 10.18832/kp2021.68.590

Radonjic, S., Maraš, V., Raičevic, J., Košmerl, T. (2020). Wine or beer? Comparison, changes and improvement of polyphenolic compounds during technological phases. Molecules, 25, 4960. https://doi.org/10.3390/molecules25214960

Steiner, E., Becker, T, Gastl, M. (2010). Turbidity and haze formation in beer - insights and overview. Journal of the Institute of Brewing, 116, 360–368. https://doi.org/10.1002/j.2050-0416.2010.tb00787.x

Szwajgier, D. (2009). Content of individual phenolic acids in worts and beers and their possible contribution to the antiradical activity of beer. Journal of the Institute of Brewing, 115, 243–252. https://doi.org/10.1002/j.2050-0416.2009.tb00376.x

Ushida, M., Suga, S., Ono, M. (1996). Improvement of oxidative flavor stability of beer - Rapid prediction method for beer flavor stability by electron spin resonance spectroscopy. Journal of the American Society of Brewing Chemists, 54, 205–211. https://doi.org/10.1094/ASBCJ-54-0205

Vanbeneden, N., Van Roey, T., Willems, F., Delvaux, F., Delvaux, F.R. (2008). Release of phenolic flavour precursors during wort production: Influence of process parameters and grist composition on ferulic acid release during brewing. Food Chemistry, 111, 83–91. https://doi.org/10.1016/j.foodchem.2008.03.029

Wannenmacher, J., Cotterchio, C., Schlumberger, M., Reuber, V., Gastl, M., Becker, T. (2019). Technological influence on sensory stability and antioxidant activity of beers measured by ORAC and FRAP. Journal of the Science of Food and Agriculture, 99(14), 6628–6637. https://doi.org/10.1002/jsfa.9979

Wannenmacher, J., Gastl, M., Becker, T. (2018). Phenolic Substances in Beer: Structural Diversity, Reactive Potential and Relevance for Brewing Process and Beer Quality. Comprehensive Reviews in Food Science and Food Safety, 17(4), 953–988. https://doi.org/10.1111/1541-4337.12352

Zavrelová, M., Psota, V., Matusinsky, P., Musilová, M., Némethová, M. (2021). Evaluation of malting quality of spring barley genetic resources from different regions of origin. Kvasny Prumysl, 67(1), 392–402. https://doi.org/10.18832/kp2021.67.392

Zhao, H., Zhao, M. (2012). Effects of mashing on total phenolic contents and antioxidant activities of malts and worts. International Journal of Food Science and Technology, 47, 240–247. https://doi.org/10.1111/j.1365-2621.2011.02831.x