Application of a hop by-product in brewing: reduction in the level of hazy-active prolamines and improved antioxidant properties of the beer

Lukáš Jelínek,* Marcel Karabin, Blanka Kotlíková, Tereza Hudcová and Pavel Dostálek

After supercritical CO₂ extraction of hops or formation of lupulin-enriched pellets (type 45), polyphenol-rich by-products remain. These have some industrial and agricultural applications (preparation of polyphenol extracts and in the production of low-bitterness beers). In this work, both materials were used in pilot-scale brewing experiments for the production of beer enriched with polyphenols. Samples of beer made from these residues underwent analyses for hazy-active prolamines, total polyphenols, phenolic monomers, antioxidant capacity, bitterness and colour. An almost identical decrease in the content (from hopped wort to final beer) of the prolamines of approximately 30% was detected in standard beer as well as in the beer prepared from the hop residue (HR 1) after supercritical CO₂ extraction. In contrast, the level of prolamines was reduced by more than 50% in the beer following addition of the hop residue (HR 2) after production of lupulin enriched pellets. Both hop materials contributed to an increase in the antioxidant capacity of the final beer by 21.5 and 11.9%, respectively. Another part of this work was aimed at the investigation of colour and bitterness changes in beers made using hop by-products. Addition of the material HR 2 into the wort caused an increase in colour and in bitterness of the matured beer by 20 and 11% rel., respectively.

Keywords: hops; colloidal stability; stabilization; antioxidant activity

Introduction

Hops (Humulus lupulus L.) is one of the most important raw materials employed in brewing. Dried cones are a bulky product, containing only about 20% w/w of valuable brewing compounds (resins, essential oils and polyphenols). For this reason, the majority of hop crops are processed into hop pellets or extracts (1).

There are two basic types of hop pellets: type 90 (normal hop pellets) and type 45 (lupulin-enriched hop pellets) (2). The first step in the production of both types of pellets is cooling of the dried cones (moisture down to 5% w/w) at −30°C and crushing with a hammer mill. Next, the hop powder (particle size 2–8 mm) is homogenized and compressed into hop pellets. In the case of lupulin-enriched pellets, separation of the lupulin fraction is achieved using a special sieving system cooled to −30°C. The sieved lupulin grains are compressed into type 45 pellets. For the preparation of hop extract, only solvents naturally present in beer (ethanol and carbon dioxide) are used. Ethanol has low selectivity, thus, in addition to large quantities of bitter acids, the raw ethanol extract also contains various polar compounds (polyphenols, carbohydrates, proteins etc.) (3); further purification is therefore necessary. Nowadays, the use of supercritical CO₂ (>7.3 MPa and >30°C) (4) as an extraction solvent is preferred, owing to its selectivity for bitter and aromatic hop compounds. Hop residues after production of lupulin-enriched pellets, or after CO₂ extraction, contain up to 1% (w/w) of α-bitter acids, while the concentration of phenolic compounds, especially flavan-3-ols and phenolic acids, can be slightly higher than in the dried hop cones.

The main cause of beer haze is the presence of flavan-3-ols (catechin, epicatechin, gallocatechin etc.) (5–7). Their oxidized forms can react with proline-rich proteins (prolamines from malt and cereals), and colloidal particles are formed (8–10). For this reason, many brewers try to extend the shelf-life of the beer by the removal of polyphenolic compounds using polyamide sorbents (polyvinylpolypyrrolidone or polyamide 6) (11–13). However, this treatment removes not only haze precursors, but also significant portions of compounds with proven antioxidant properties and potential cancer chemopreventive activities (14). Stabilized beer usually has reduced antioxidant capacity and is prone to the formation of sensory active compounds, formed by oxidative reactions (15–17). Several previous studies have shown that a controlled increase in the concentration of some polyphenols can improve the shelf-life of this beverage. Aerts and colleagues (18) proved the positive effect of gallotannins (10 g/hL in brewing water and 5 g/hL in sparging water) on the colloidal stability of beer. These authors also established...
increased brewhouses yields and improved lautering performances (faster drop of extract and run-off rate during lautering) in sweet worts by the addition of galloptannins. Pöschl and colleagues (19) used polyphenol extracts from the hop cultivars, Saaz and Hallertauer Tradition, to improve the colloidal stability of the beer. Polyphenol-enriched beers had a significantly reduced content of tannin-precipitable nitrogen compared with standard beer. Haze formation was also slightly lower in the samples containing the polyphenol extract, although these beers contained increased levels of both haze-active flavan-3-ols and phenolic acids.

Waste materials such as brewery waste streams (20,21) or vegetative waste material of hop pellets (22) are rich and currently underused sources of phenolic antioxidants, which could be reused for many industrial or pharmaceutical purposes. In this manuscript, hop by-products (residues from the production of lupulin-enriched pellets and from CO₂ extraction) were used for the pilot-scale preparation of special polyphenol-enriched beers. The main aim of this work was to reduce the level of haze-active prolamines through precipitation with hop flavan-3-ols (analogous to galloptannin stabilization) during beer maturation (23). Analyses of bitterness, colour and antioxidant properties of polyphenol-enriched beers are also presented in this work.

Materials and methods

Hop material

Vital hop (pellets type 90) and hop residue (HR) 1 (pellets derived from CO₂ extraction of Vital hop) were kind gifts from Chmelárství, družstvo Žatec (Czech Republic). Saaz hop (dried cones) and hop residue HR 2 (pellets derived from lupulin-enriched pellets of Saaz hop) were kind gifts from the Bohemia Hop Co. Ltd (Czech Republic). All samples were stored at 8°C until needed.

Hop analyses

Analyses of α-bitter acids in hop samples were performed according to EBC method 7.7 (24). Essential oils were steam-distilled using 100 g of crushed hop material (3 h). The raw distillate was purified in a separating funnel with diethylether (50 mL for 2 mm). The organic phase was dried overnight with water-free Na₂SO₄. The organic solvent was removed by evaporation under vacuum to a constant weight (25).

Hot water extracts for the analyses of total polyphenols and antioxidant capacity were prepared as follows: 10.0 g of crushed hop material (3 h). The raw distillate was purified in a separating funnel with diethylether (50 mL for 2 mm). The organic phase was dried overnight with water-free Na₂SO₄. The organic solvent was removed by evaporation under vacuum to a constant weight (25).

Total polyphenols in the hot water extracts, worts and beers were analysed according to EBC method 9.11 (24). Antioxidant capacity in the hot water extracts, worts and beers was measured with the spectrophotometric method using DPPH (1, 1 diphenyl-2-picyrylhydradrazil) according to Kaneda and colleagues (26). Results are presented as a percentage of decolourization of the DPPH solution (ACDPPH) calculated using the following formula:

\[ AC_{DPPH}(\%) = \frac{(\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}})}{\text{Absorbance}_{\text{blank}}} \times 100 \]

The analysis of haze-active polyphenol monomers [(+)-catechin, (−)-epicatechin and ferulic acid] from hop materials was carried out according to Jelinek and colleagues (25). Crushed hop material (5 g) was extracted with dichloromethane (2 × 100 mL for 1 h) to remove the hop bitter acids. The residual solid sample was extracted with 70% v/v acetone (3 × 50 mL, 30 min). The organic solvent (acetone) was evaporated and the residue was filtered up to the volume of 50 mL with distilled water. A 1 mL sample was passed through a 0.2 µm cellulose acetate filter and subjected to HPLC analysis.

Wort and beer production

Three different brewing experiments were performed in the 100 L pilot brewery. Pilsen malt (8.2 kg) was mashed with 50 L of water according to the followed scheme: 38°C for 25 min; 65°C for 35 min; 75°C for 15 min; and 78°C for 15 min. The temperature gradient rate was ~1°C/min. The worts containing different hop materials were boiled for 90 min:

- CO₂ extract (12 g) containing 36.9% (w/w) of α-bitter acids (standard beer);
- Saaz hop (pellets type 90; 69 g) containing 4.8% (w/w) of α-bitter acids + 140 g of HR 1 containing 0.8% (w/w) of α-bitter acids (beer + HR 1);
- Saaz hop (pellets type 90; 69 g) + 187 g of HR 2 containing 0.6% (w/w) of α-bitter acids (beer + HR 2).

Standard beer was hopped with only the polyphenol-free extract for a better demonstration of the impact of hop polyphenols on colloidal and antioxidant properties of beer. On the other hand, aromatic hop with a high content of phenolic compounds was used to prepare both of the HR-beers. The main goal of this hopping step was to prepare worts with a high content of polyphenols, which would be capable of reacting with haze-active proteins during maturation.

The content of the total α-bitter acids in all three hopped worts was ~4.4 g. The original gravity of the hopped worts was ~11.6°P. The time of fermentation and maturation were 7 days (10°C) and 26 days (2°C) respectively.

Wort and beer analyses

The bitterness and colour were determined according to EBC methods 9.6 and 9.8 respectively (24). In the case of phenolic monomers [(+)-catechin, (−)-epicatechin and ferulic acid], 25 mL of wort or degassed beer was extracted with ethyl acetate twice (25 mL for 2 min). The raw extract was evaporated under vacuum and the solid residue was dissolved in 1 mL of 50% methanol, and adjusted to pH 3 with formic acid. The sample was then passed through a 0.2 µm PTFE membrane and analysed by HPLC.

Haze-active polypeptides concentrations (prolamines expressed as gliadin) were determined according to Dostálek and colleagues (27). A commercial kit, Ridascreen® Gliadin Competitive, based on the RS monoclonal antibody, was used for the prolamine determination in wort and beer. The limit of detection for wort and beer was 1.36 mg gliadin/L. The limit of quantification for wort and beer was 5 mg gliadin/L.

HPLC analyses of polyphenols monomers

The separations of polyphenol monomers were carried out using the Agilent Eclipse XDB-C₁₈ column (5 µm, 4.6 x 150 mm) in an
Agilent 1100 HPLC system equipped with a diode array detector. The mobile phase consisted of solvents A (methanol adjusted to pH 3 with formic acid) and B (deionized ultra-pure water adjusted to pH 3 with formic acid). The separation was performed using the following gradient conditions: from 95 to 90% of solvent B in the first 5 min; from 90 to 65% in the next 20 min; from 65 to 10% in the next 5 min; and from 10 to 95% in the final 5 min. The duration of analysis was 35 min and the flow rate was maintained at 1 mL/min; the injected volume was 20 μL and the column temperature was 30°C. The wavelengths used for detection were 280 nm for catechin and epicatechin and 320 nm for ferulic acid. Quantitative analysis was carried out using an external calibration method.

Data presentation and statistical analyses

All measurements were performed in triplicate and are presented as a mean ± standard deviation. Data were analysed by Statistica 10.0 and results were processed by analysis of variance (ANOVA) followed by Tukey’s honest significant difference (HSD) test (p < 0.05).

Results and discussion

Characteristics of the hop by-products

Hop by-products are the waste material produced during the processing of dried hop cones or pellets into forms rich in α-bitter acids (hop extract or lupulin-enriched pellets). In both cases, by-product is the finely ground hop powder, which has been compressed into pellets for ease of handling. These materials usually contain only small amounts of hydrophobic products secreted by the lupulin glands (resins and oils), while the polyphenol content remains the same or is slightly increased in comparison with the original material. Figure 1 shows the concentration of α-bitter acids, total oils and total polyphenols in the hop by-products and in their original hop materials.

Vital is a Czech bitter hop variety registered in 2008 (28). This hop is commonly used for the production of bitter acid extracts. For the purpose of this work, carbon dioxide extraction was carried out on the type 90 pellets made from Vital hop. The residue after this process is a bitter-free material and was named HR 1 for identification in this paper. Saaz is the most widespread Czech fine-aroma variety registered in 1952 (Osvald clones 31 and 72) (29). This cultivar is mainly used for the production of type 90 or 45 pellets. In this work, the dried cones of Saaz hop and the residue after production of lupulin-enriched pellets (named HR 2) were compared.

As can be seen in Fig. 1(a), most of the α-bitter acids in the original hop matrix were removed by extraction or sieving. Both hop by-products contained up to 1% w/w of α-bitter acids. A considerable decrease in content was also observed in the case of the total oil (Fig. 1b). While the original Vital and Saaz hop materials contained 700 and 300 mg/100 g, respectively, of total oil, the residues HR 1 and HR 2 contained only 50 and 70 mg/100 g respectively. In the case of total polyphenols (Fig. 1c), hop residue HR 1 contained slightly lower levels of these compounds than the original hop material (Vital pellets). When the Saaz hop and HR 2 were compared, however, the latter contained almost twice the concentration of polyphenols than the Saaz hop. This phenomenon can be explained by the loss of the non-polyphenolic hop fraction (lupulin fraction) from the original hop material. The residue has a lower weight, but the actual amount of polyphenols remains unchanged. The large increase in polyphenol concentration in HR2 can also be explained by the low temperature (~30°C) during the hop cone processing step into the lupulin-enriched pellets. Unlike the CO₂ extraction (temperature ~30°C), low temperature prevents the oxidative loss of polyphenols.

Our results are in contradiction to those of Kowalczyk at al. (30). The authors state that most of the phenolic compounds from hop cones pass into pellets (type 45) during processing. On the other hand, Baranowski (31) point out that only half of the original content of the polyphenols from pellets type 90 pass into type 45 pellets.

In recent years, many authors have shown a correlation between the concentration of total polyphenols in the hop matrix and antioxidant capacity, where polyphenol-rich cultivars exhibit better antioxidant properties (32,33,22). In the current work, the antioxidant capacity of the HR materials and their original hop materials were compared (Fig. 2). Although the Vital pellets contained a slightly higher concentration of total polyphenols than HR 1, higher antioxidant capacity was detected in the latter. This unusual behaviour could be caused by the synergistic effect between phenolic compounds, or the loss of
compounds with low antioxidant activities, during hop treatment. An entirely different result was seen with Saaz hops and HR 2. A high concentration of phenolic compounds in HR 2 is reflected by the 15% increase in antioxidant capacity.

Table 1 shows the concentration of haze-active polyphenols (catechin, epicatechin and ferulic acid) found in hop residues and in the original hop material. Removal of hop resins and oils resulted in an increase in the concentration of phenolic compounds in both hop residues. The most pronounced increase was observed in HR 1 (+44.5 mg/100 g for catechin, +14 mg/100 g for epicatechin and +1.1 mg/100 g for ferulic acid).

As described above, both hop materials contained only small amounts of bitter acids and essential oils. Therefore, it can be assumed that their use in hop boiling would not bring about an undesirable increase in bitterness or aroma changes in the beer. On the other hand, a high content of total polyphenols can considerably improve the antioxidant properties of the final beer. The high concentration of haze-active phenolic compounds from HR 1 and HR 2 could trigger a precipitation reaction with beer prolamine, which could result in severe haze formation during maturation. However, this turbidity could be subsequently removed by filtration.

Characteristics of hopped worts and beers
The amounts of total polyphenols in the hopped worts, beers after main the fermentation and final beers are shown in Fig. 3. The additions of HR 1 and HR 2 to the wort caused a significant increase in the concentration of total polyphenols (+154 and +297 mg/L, respectively). From hopped wort to matured beer, a nearly equal decrease in the concentration of total polyphenols was detected in the beers with HR 1 and HR 2 (-135 and -122 mg/L, respectively). Despite these losses, beer with the addition of HR 2 contained almost three times more total polyphenols than the standard beer.

As expected, the beers with the added hop by-product also contained a considerably higher level of flavan-3-ols, catechin and epicatechin (Fig. 4a and b). The increase in catechin content in the worts was approximately 2.5 mg/L for the wort with HR 1 and approximately 7.2 mg/L for the wort with HR 2. The catechin

Table 1. Major phenolic precursors of protein-polyphenols haze found in hop materials

<table>
<thead>
<tr>
<th>Haze-active polyphenols (mg/100 g)</th>
<th>Catechin</th>
<th>Epicatechin</th>
<th>Ferulic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital (pellet type 90)</td>
<td>97.0 ± 6.0</td>
<td>67.4 ± 5.6</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>HR 1</td>
<td>141.5 ± 9.8</td>
<td>81.4 ± 3.3</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>SAAZ (dried cones)</td>
<td>307.8 ± 10.8</td>
<td>51.4 ± 5.7</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>HR 2</td>
<td>331.0 ± 10.1</td>
<td>52.3 ± 4.6</td>
<td>2.7 ± 0.1</td>
</tr>
</tbody>
</table>
level in beer with added HR 1 was relatively stable during the entire process (decrease by 1.2 mg/L from hopped wort to mature beer). On the other hand, more than 4 mg/L of this compound was lost during the fermentation and maturation of beer with HR 2.

Changes in epicatechin levels were analogous to those observed for catechin. The concentration of epicatechin in hopped worts with the addition HR 1 and HR 2, in comparison to standard beer, increased by 1.4 and 2.4 mg/L respectively. Unlike catechin, the content of this polyphenol decreased significantly in both investigated beers. The addition of hop residues had a minimal effect on the concentration of ferulic acid in wort (Fig. 4c). Beer and wort with HR 1 retained similar concentrations of this phenolic acid to standard beer (~2 mg/L). With the addition of HR 2, the ferulic acid content in the hopped wort increased by 0.7 mg/L. During the fermentation and maturation process, this concentration dropped from 2.9 to 1.5 mg/L.

The concentration of flavan-3-ols and ferulic acid in beer has a fundamental effect on haze formation. A decrease in the amount of these compounds during fermentation and maturation can be the result of precipitation reactions with haze-active peptides.

Prolamines from barley and malt are the main components of beer colloidal haze. Their molecules are characterized by high levels of proline (34) and a repeated amino acid sequence, QQPFF (35). In current practice, the selective removal of these proteins is achieved using silica gels (36). Figure 5 shows the concentration of prolamines in standard beer and in beer with the addition of hop residues during fermentation. Although the wort prepared from hop material HR 1 contained slightly more prolamines than standard beer, the decrease in content of these substances during fermentation and maturation was almost identical (by ~6.7 mg/L). On the other hand, with HR 2, there was a significant reduction in the content of prolamines in the final beer (>12 mg/L). It can be assumed that this phenomenon was caused by the high concentrations of haze-active flavan-3-ols present in the HR 2, which were able to precipitate the prolamines.

The addition of polyphenol-rich hop by-products to the wort had a significant impact on the antioxidant properties of beer (Fig. 6). The addition of either hop residue increased the antioxidant capacity of the wort (+15 and +11.4%) and matured beer (+21.5 and +11.9%) in comparison to the standard beer. An interesting result was obtained in the case of beer prepared from HR 1. Although this material contained a lower concentration of phenolic compounds (in comparison with HR 2), the antioxidant capacity of the beer prepared from it was slightly higher. A possible explanation of this phenomenon might be the precipitation of a large amount of phenolic antioxidants with haze-active prolamines during the fermentation and maturation processes.

For our investigation, it was necessary to understand how the use of a hop by-product could affect the basic characteristics of beer, such as colour and bitterness. The results of these analyses are given in Table 2. HR 1 had almost no effect on the colour or bitterness, whereas beer made from the HR 2 hop material was characterized by slightly higher values for both of these parameters (increase in colour ~20% rel.; increase in bitterness ~11% rel.). The HR 2 contribution to the values of colour and bitterness may be considered unacceptable by many breweries. However, these defects could be easily eliminated by processing the material into the form of a polyphenol-rich extract. The method for industrial-scale extraction of hop polyphenols, as proposed by Biendl (3), could be used for this purpose.

**Conclusions**

In this manuscript, the composition of hop waste materials (residues from the production of lupulin-enriched pellets and CO₂ extract) was compared. It has been shown that these residues contain only a small amount of α-bitter acids and are rich sources of polyphenols, especially haze active flavan-3-ols. It was also confirmed that these materials have very strong antioxidant properties. The use of polyphenol-rich hop by-products during wort boiling resulted in a reduced level of haze-active prolamines in the matured beers. Beer made from these hop residues had an increased antioxidant capacity, while changes in colour and bitterness were minimal with the HR 1 material. Beers made from HR 2 had a slight increase in both the colour

---

**Table 2.** Colour and bitterness of standard beer and beers made from hop residues

<table>
<thead>
<tr>
<th>Colour (EBC)</th>
<th>Bitterness (EBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard beer</td>
<td>9.7 ± 0.8</td>
</tr>
<tr>
<td>Beer + HR 1</td>
<td>9.6 ± 0.4</td>
</tr>
<tr>
<td>Beer + HR 2</td>
<td>11.6 ± 0.3</td>
</tr>
</tbody>
</table>

---

**Figure 5.** Concentration of haze-active prolamines from hopped worts to matured beers in standard samples, samples + HR 1 and samples + HR 2.

**Figure 6.** Concentration of haze-active prolamines from hopped worts to matured beers in standard samples, samples + HR 1 and samples + HR 2.
and bitterness levels. The use of hop by-products cannot substitute for silica gel stabilization, but it could considerably reduce the cost of beer stabilization.

Acknowledgements
This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (KONTAKT ME101110) and Ministry of Agriculture of the Czech Republic (Project Q11118053).

References