Dry-Hopping: the Effects of Temperature and Hop Variety on the Bittering Profiles and Properties of Resultant Beers

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Dry-Hopping: the Effects of Temperature and Hop Variety on the Bittering Profiles and Properties of Resultant Beers

This paper reports the effects of dry-hopping at 4 and 19 °C for a low alpha versus high alpha hop variety on the resulting profiles of non-volatile hop acids (humulinones, iso-α-acids, α-acids). In a dry-hopping study conducted over 2 weeks, we found a significant increase in humulinone concentration driven principally by hop alpha acid content and the duration of dry-hopping. Conclusive evidence of iso-α-acid losses during dry-hopping (by adsorption onto spent hops) is presented, in addition to a significant increase in α-acid concentrations, which was observed only for beers dry-hopped at 19 °C with the high alpha hop variety. Measured beer parameters (especially at 19 °C) revealed an increase in pH, ABV (%), and a decrease in beer density during dry-hopping – from which we conclude that further attenuation of beer occurred during dry-hopping. The polyphenol content of beers was found to increase substantially with dry-hopping time, whilst both temperature and hop variety were found to be significant factors determining the amounts of polyphenols extracted. Finally, analysis of the spent hop slurry (recovered after 14 days of dry-hopping) confirmed that the residual content of hop acids (α-acids, their oxidised derivatives and polyphenol content), makes these materials – currently treated as waste – of potential value for re-use in the brewing process.

Descriptors: dry-hopping, humulinones, 'hop-creep', bitterness quality, spent hops, hop polyphenols

1 Introduction

There is no doubting the significant contribution of dry-hopping to the renaissance of craft beer and the subsequent boom in sales of craft beers around the world. On the face of it, dry-hopping represents a relatively simple means of improving the flavour of beer; for this, brewers add between 2–12 g/L of hops in the form of cones or pellets into beer during fermentation or conditioning for periods ranging from several days to weeks [23]. The added hops can be left in the beer with no agitation (static dry-hopping) or with agitation, using a pump or CO_2 for example (dynamic dry-hopping). Brewers can also add hop oil essences to beer to create specific flavour characters that mimic dry-hopped flavours in their product [6]. Although the basics of dry-hopping as described above are agreed, there is no common approach to dry-hopping within the brewing industry; with most breweries adopting dry-hopping practices based on product line, brewhouse volume and processing capabilities. The addition of hops to beer in the cold stages of processing results in a cascade of changes to beer quality – the implications of which most brewers and researchers-alike are still attempting to decipher. Brewers often have to consider several factors in relation to dry-hopping in order to produce beers with consistent hoppy flavour. These include hop variety selection and harvest date, rate of addition, oil content of selected hop, dry-hopping temperature, and whether to adopt a dynamic or static dry-hopping process [4, 11, 23]. Even the alcohol content of the beer to be dry-hopped must be considered, in order to prevent the extraction of unwanted hop vegetative materials into the finished product due to the solvating power of ethanol [20]. The duration of dry-hopping (contact time) is another crucial factor to control the balance of compounds extracted from hops into beer. Due to the hydrophobicity of some hop aroma compounds, dry-hopping for prolonged periods can cause their partitioning out of beer back onto spent hops. The losses or reduction in concentration of important marker aroma compounds such as linalool has been reported after prolonged dry-hopping [23]. Both high alcohol content and prolonged contact time can increase the extraction of unwanted vegetative materials, which often lead to the generation of 'grassy' flavours in dry-hopped beers.

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The increase in aroma perceived in dry-hopped beers versus conventionally hopped beers is thought to be due to elevated levels of several volatile terpene compounds, hydrocarbons and their derivatives e.g. linalool, myrcene, humulene, β-Citronellol and geraniol [11, 22, 23]. The labile nature of these compounds means that they are rarely present in beers that are not dry-hopped, because they are easily lost to evaporation during wort boiling or fermentation [6]. Furthermore, the presence of yeast during dry-hopping adds an extra level of complexity to this process. Some researchers have reported the biotransformation of certain volatile hop compounds during and post fermentation (maturation), e.g., in the conversion of geraniol into β-citronellol [21]. This means that brewers must also decide whether to totally remove yeast from beer before dry-hopping. The presence of yeast during dry-hopping may offer other benefits – suspended yeast can metabolise dissolved oxygen during dry-hopping, thereby protecting beer from oxidation during the process [23].

Perhaps one of the unintended consequences of dry-hopping is the effect this process has on perceived beer bitterness. Several studies have shown an increase in both measured analytical (BU) and perceived bitterness in dry-hopped beers [1, 12, 16, 23]. These observations have been explained by the presence of oxidized α-acids (humulinones), which are highly prevalent in dry-hopped beers. Humulinones have been reported in leaf and pellet hops [14]. They are readily soluble in beer and have a bitterness intensity of 66 % relative to iso-α-acids [1]. The second explanation is that volatile aroma compounds in hops can enhance the perception of bitterness intensity, as well as modify bitterness quality, particularly at low BU levels - as was demonstrated through the addition of hop oils to beers of different analytical BU [18]. This observation is most likely due to a multimodal interaction between the perception of taste and aroma. The extraction of hop polyphenolic compounds into beer during dry-hopping is another contributory factor to increased bitterness perception in dry-hopped beers; these compounds are widely accepted to contribute both bitterness and astringency to beer depending on their molecular mass [3, 15, 19]. Dry-hopping has also recently been reported to affect beer parameters, e.g. beer pH, foam and International Bitterness Unit (IBU) readings determined by the spectrophotometric method [12–14]. To date, most of the available studies on dry-hopping have focused on volatile hop aroma compounds and the dynamics of transfer of these compounds into beer during dry-hopping. Consequently, this study was designed to investigate the behaviour of non-volatile compounds which affect perceived bitterness during dry-hopping, as influenced by temperature and time for two hop varieties. A time-course experiment lasting 14 days was designed to investigate the effects of dry-hopping with a low alpha hop (Hersbrucker, 3 % α) and high alpha hop variety (Zeus, 17 % α) at both 4 & 19 °C, on the composition of hop acids (humulinones, iso-α-acids, α-acids) in dry-hopped beers, as well as in the spent hops left after dry-hopping. We further investigated the impacts of dry-hopping on the total polyphenol content of dry-hopped beers, and several beer parameters, including pH, ABV (%) and beer density in the resulting dry-hopped beers.

Fig. 1 Dynamic dry-hopping apparatus

2 Materials and methods

2.1 Hops

The hops selected for this study (T90 pellets of the varieties Hallertau Hersbrucker and Zeus) were purchased from Simply Hops (Paddock Wood, Kent, UK). The hops were of 2015 crop year and contained the following amount of oil and α-acids, respectively: Hersbrucker (0.5–1 mL /100 g, 3 %) and Zeus (2.5–3.5 mL /100 g, 17 %). The precise α-acid content was determined in a previous study [17].

2.2 Hop acid standards and chemicals

Iso-α-acid standard (ICE-3) containing trans-isocohumulone, trans-isohumulone, trans-isoadhumulone (62.3 % w/w) was purchased from Labor Veritas Co. (Switzerland). Humulinones were synthesised in-house using the method previously detailed [19]. Extraction chemicals (methanol, dichloromethane) and those used in the polyphenol assays were purchased from VWR (UK).

2.3 Beer production

A 50 L all-malt brew was produced from lager malt in the pilot brewery of the University of Nottingham. A single infusion mash protocol at 68 °C was adopted. The wort was hopped with East Kent Goldings hops (6.8 % α-acids) to achieve a target of 20 bitterness units (BU). Boil duration was 60 min and the post-boil gravity was 1.040 (10 °P). The hopped wort was cooled to 15 °C and transferred into 4 separate 30 L capacity FastFerment™ conical fermenters, each containing 11 L of wort. The wort in each fermentation vessel was fermented with Saflager S-23 dried yeast (11 g) from Fermentis, with fermentation conducted at 18 °C for 7 days. The resultant young beer was cooled to 3 °C for 3 days to give base beers with a final gravity of 1.008 (2 °P). The alcohol content of the base beer was 4.32 % ABV and pH was 3.82. At the end of maturation, the sedimented yeast collected in a collection bulb at the bottom of each fermenter was discarded (Figure 1). All of the fermenters were left at room temperature to equilibrate for 4 h before being set up for dry-hopping (Figure 1).

2.4 Dynamic dry-hopping set-up

T90 pellet hops (of either Hersbrucker or Zeus variety) were added to the fermentation vessels at a rate of 4 g/L and dry-hopping took place in temperature controlled rooms set at 4 and 19 °C. A dynamic dry-hopping set-up was mimicked by inserting an overhead stirrer fitted with a paddle into the fermenters. The headspace of each vessel was purged with N_2 after hop addition (to displace O_2), before the commencement of dry-hopping. The stirrers in all four fermenters were set to rotate at 200 RPM with the aid of a tachometer (Figure 1).

2.5 Sample collection time points

Samples were collected over a 14-day period. The first time point samples were collected prior to the addition of hops and denoted Day 0 samples. The base beer had a total bitterness concentration 18.3 mg/L of iso-α-acids by HPLC. Subsequent samples were collected a day later (Day 1), 3, 7, 10 and 14. Samples were collected from the bottom of the fermenter (in the collection bulb) with the aid of the isolation valve.

2.6 Determination of hop acids in fresh and spent hops

A small quantity of each hop variety was first pulverised with a blender. Then 1 g of each hop was transferred into a 50 mL centrifuge tube. Methanol (10 mL) was added to extract hop acid compounds. Samples were extracted on a roller bed for 1 h, and upon completion centrifuged at 5000 RPM for 5 min to aid phase separation. An aliquot of the supernatant was filtered through a 0.20 µM polytetrafluoroethylene (PTFE) membrane syringe filter in readiness for hop acid analysis by RP-HPLC (as below).

For spent hops, a slurry of spent hops was recovered from each fermentation vessel at the end of dry-hopping (Day 14) and filtered to dryness through a crucible under vacuum. One gram of the dry spent hop material was then subjected to extraction as described above.

2.7 Determination of hop bitter acid compounds

The separation of hop acids (humulinones, iso-α-acids, α-acids, β-acids) and their relative concentrations in hops and beer was determined by RP-HPLC as previously described in Oladokun et al. (2016). Hop acids were extracted from beer as follows: beer sample (5 mL) was acidified with orthophosphoric acid (100 uL) and extracted into isooctane (10 mL) on a roller bed for 30 min. The isooctane extract was subsequently transferred into a glass tube and evaporated to dryness under nitrogen. The residue was reconstituted in acetonitrile (2 mL) and analysed by HPLC. Samples were analysed in triplicate and hop acid concentrations were acquired from calibration curves generated from external hop acid standards prepared in the range of 1, 5, 10, 20, 40, 60 mg/L. Humulinone standards were prepared in the concentration range of 1, 10, 20, 40 and 80 mg/L.

2.8 Total polyphenol content

The total polyphenol content of dry-hopped beers was determined according to the standard beer ASBC Beer-35 method. Beer sample (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1 %) and ethylenediamine tetra acetic acid (EDTA, 0.2 %) (8 mL) in a 25 mL volumetric flask, then ferric ammonium citrate (3.5 %, 0.5 mL) was added, followed by ammonium hydroxide solution (33.3 %, 0.5 mL) with mixing after each addition. The solution was made up to mark with Reverse Osmosis (RO) water and left to stand at room temperature for 10 min. The absorbance of the solution was taken at 600 nm and multiplied by 820 to give the total polyphenol content in beer (mg/L). The assay was conducted in triplicate for each sample.

2.9 Total polyphenol content of fresh and spent hops by Folin-Ciocalteau method

Total polyphenol content of spent hops was determined using the Folin-Ciocalteau colorimetric assay. Pulverised hops (1 g each) were first extracted into dichloromethane (15 mL) on a roller bed for 30 min (separately twice), in order to remove hop acid compounds. The dichloromethane extract was discarded and the hop residue was subjected to further extraction in 70 % methanol in water (10 mL) for 30 min on a roller bed. Extraction was repeated once more and the combined extracts were used for total polyphenol content determination in hops. Recovered spent hop slurry was dried as described in Section 2.6 before extraction with dichloromethane and 70 % methanol in water as described above. For the colorimetric assay, 20 µL of the methanolic extract was combined with water (1.58 mL) and Folin-Ciocalteau reagent (100 µL) in a cuvette. The mixture was mixed well and left for 5 min to react. Saturated sodium carbonate (300 µL) was added and the whole mixture was mixed and left at room temperature for 2 h. Absorbance readings were taken for the samples at 765 nm. Total polyphenol concentration was determined from a gallic acid calibration curve of 0, 100, 150, 250, 500 mg/L, and expressed as gallic acid equivalent per litre of hop polyphenol extract (mg of GAE/L of extract).

2.10 Determination of beer density, % ABV and pH

The alcohol content, density and gravity of beer samples were measured using an Anton Paar DMA 4500 coupled to an Alcolyzer Plus instrument (Anton Paar, Austria). Beer pH was determined with a pH meter (Metler Toledo). All measurements were made in triplicate.

2.11 Statistical analysis

Statistical analysis was carried out with XLSTAT version 2017 (Addinsoft, Paris). A 3-way (hop variety, temperature and time) Analysis of Variance (ANOVA) test was conducted to determine significant impacts ($P < 0.05$) of these dry-hopping factors on each of the measured analytical properties of the resulting beers.

3 Results and Discussion

3.1 Impacts of dry-hopping on the resulting profile of hop acid compounds in beer.

3.1.1 Humulinones

The concentrations of humulinones measured in beers dry-hopped with Zeus and Hersbrucker are presented in figures 2A and 2B respectively. For the beers dry-hopped with Zeus, (Figure 2A), a significant increase ($p < 0.05$) in the concentration of humulinones was observed at both temperatures investigated over the course of dry-hopping. The largest increase in humulinone concentration (58 mg/L) was observed after just 24 h of dry-hopping at 19 °C; and although at 4 °C, the concentration of humulinones also increased to 37 mg/L after 24 h, the temperature at which dry-hopping was conducted was not a significant factor ($p > 0.05$) determining the concentration of humulinones in dry-hopped beers. The maximum

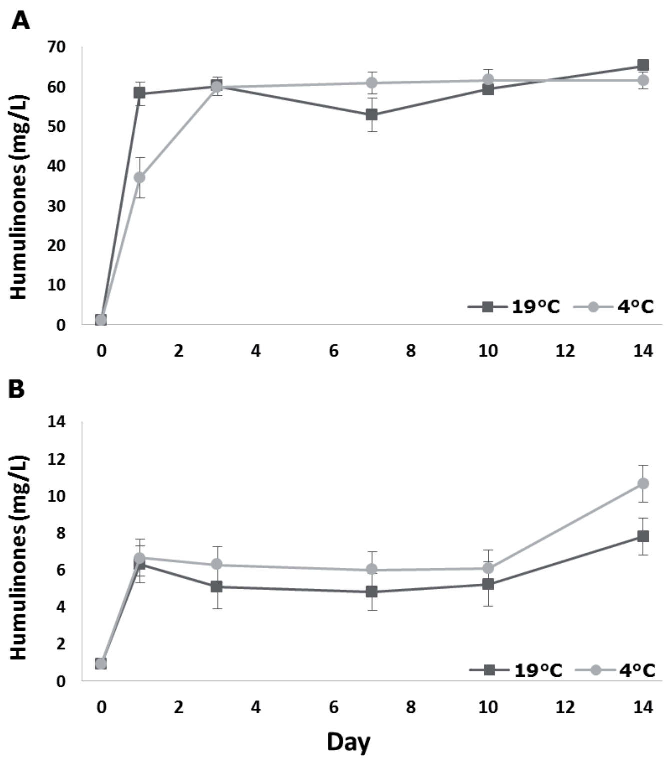

Fig. 2 Changes in the concentration of humulinones over the course of dry-hopping at 4 and 19 °C with (A) high alpha Zeus and, (B) low alpha Hersbrucker

concentration of humulinones measured in the beers dry-hopped with Zeus did not change significantly ($p > 0.05$) after the third day of dry-hopping.

In beers dry-hopped with the low α-acid hop variety (Hersbrucker in Figure 2B), both temperature and duration of dry-hopping were significant factors ($p < 0.05$) in the concentration of humulinones found in the dry-hopped beers. The concentration of humulinones increased significantly over 14 days, with higher concentrations observed at lower dry-hopping temperatures. Maximum concentrations of 11 and 8 mg/L of humulinones were reached in the beers after 14 days of dry-hopping at 4 and 19 °C, respectively. The greater levels of humulinones found in the beers dry-hopped with Zeus, relative to Hersbrucker indicates that the formation of humulinones may be linked to levels of α-acids present in the hop used for dry-hopping. Indeed, hop variety was found to be a significant factor ($p < 0.05$) in the concentration of humulinones observed in the dry-hopped beers, with greater concentrations of humulinones attained in beers dry-hopped with Zeus hops. This observation is in contrast to the similar levels of humulinones measured in the hops themselves prior to dry-hopping (~ 0.29 %w/w) which further suggests that concentrations of humulinones in dry-hopped beers are not solely related to their extraction from hops. The dynamic dry-hopping process adopted may well have contributed to greater humulinone formation (from α-acids) compared to a static dry-hopping process. These results therefore suggest that the formation of humulinones from α-acids during dry-hopping is possible, depending on the adopted dry-hopping procedure, temperature and α-acid content of hops. To understand the levels of humulinones in commercial beers, a Popular IPA brewed in the UK was purchased and analysed by HPLC. The selected beer is marketed as a 40 IBU beer that is dry-hopped at 10 g/L, with six different aroma hop varieties. HPLC analysis revealed that this beer contained 35 mg/L of humulinones, 26 mg/L of iso-α-acids and 3.21 mg/L of α-acids (Figure 3). Thus, the concentration of humulinones in this particular beer was in fact higher than that of iso-α-acids. Based on a bitterness contribution of 66 % from humulinones, these compounds would contribute a calculated bitterness of 23.1 mg/L to yield a total bitterness value of 49.1 mg/L (humulinones (ppm) x 0.66 + iso-α-acids (ppm)). This represents a 47 % contribution from humulinones to the total bitterness of this dry-hopped beer, and further reinforces the significant impact of humulinones in offsetting the reduction in bitterness encountered due to losses of iso-α-acids during dry-hopping.

3.1.2 Iso-α-acids

Iso-α-acids represent the major source of beer bitterness and are derived from the isomerisation of α-acids. This thermally driven process occurs traditionally on the hot side of the brewing process (in the kettle) as opposed to during dry-hopping. Losses of iso-α-acids in dry-hopped beers have been reported [8, 13], and were confirmed in the present study (Figure 4), with significant ($p < 0.05$) reductions in iso-α-acid concentrations being observed during dry-hopping with both hop varieties (Zeus and Hersbrucker). Our results further showed that the principal drop in iso-α-acid concentrations occurred after the first day of dry-hopping, especially at 19 °C in both hop varieties studied (Figure 4A & B).

In the beers dry-hopped with Zeus at 19 °C, there was no further significant ($p > 0.05$) drop in the concentration of iso-α-acids over the course of dry-hopping after 24 h; whilst a gradual decrease in the concentration of iso-α-acids was observed at 4 °C. For both hop varieties, the concentration of

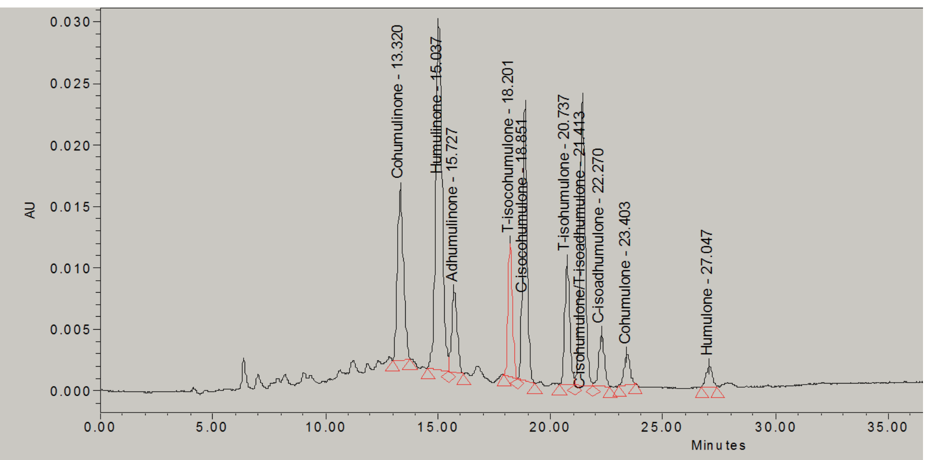

Fig. 3 Hop acid profile of a commercial craft beer produced in the UK. Humulinones: cohumulinone (13.32), humulinone (15.03), adhumulinone (15.72); iso-α-acids: trans-isocohumulone (18.20), cis-isocohumulone (18.85), trans-isocohumulone (20.73), cis-isocohumulone & trans-isoadhumulone (21.41), cis-isoadhumulone (22.27; humulones: cohumulone (23.40), humulone (27.04) and adhumulone (28.03)

iso-α-acids at the end of the 14-day dry-hopping period was not dependent on the dry-hopping temperature. Overall, the significant loss ($p < 0.05$) of iso-α-acids observed was proportionately greater with Zeus as compared to Hersbrucker (there was a 50 % drop in iso-α-acid concentration in beers dry-hopped with Zeus compared to 28 % in Hersbrucker).

3.1.3 α-acids

Although some studies have reported an increase in the α-acid concentration of dry-hopped beers [12], the overall levels of α-acids found in beer is also dependent upon upstream processing, e.g. vigour and duration of boil in the kettle, as well as the type of hop products utilised for bittering. For example, beers bittered with pre-isomerised hop products do not contain residual α-acids, whilst beers that have been conventionally bittered in the kettle with hop cones or pellets [19] retain residual concentrations of non-isomerised α-acids. Post-dry-hopping concentrations of α-acids are presented in figures 5A and B. A significant increase ($p < 0.05$) in the α-acid content of the dry-hopped beers was only observed in the beers dry-hopped with high alpha Zeus at 19 °C. This increase was gradual over the course of dry-hopping, with a smaller increase observed at the lower dry-hopping temperature of 4 °C (Figure 5A). For the low alpha variety (Hersbrucker) shown in figure 5B, there was no significant ($p > 0.05$) increase in the concentration of α-acids in the dry-hopped beers over the 14 days of dry-hopping. In contrast to Zeus, the temperature at which dry-hopping was conducted did not make a significant ($p > 0.05$) difference to the levels of α-acids found in the beers dry-hopped with Hersbrucker.

These results therefore suggest that the content of α-acids in dry-hopped beers may be associated with the α-acid content of the hop variety used for dry-hopping, and therefore the use of high alpha hops for dry-hopping at higher temperatures may yield higher residual α-acids in the final product. Furthermore, hop variety was found to be a significant factor ($p < 0.05$) in the concentration of α-acids in dry-hopped beers; higher α-acid concentrations were observed in Zeus compared to Hersbrucker. Although α-acids at concentrations below 14 mg/L are thought not to contribute substantially to beer bitterness [7], a considerable impact on perceived bitterness of beer from the combination of humulinones, iso-α-acids and elevated residual α-acids cannot be discounted. The concentration of β-acids did not increase in the dry-hopped beers presumably due to their documented poor solubility in beer.

3.2 Impact of dry-hopping on the total polyphenol content of beer

3.2.1 Hersbrucker

The total polyphenol content (TPC) of the dry-hopped beers was determined, and for the beers dry-hopped with Hersbrucker, the result is presented in figure 6A. In the base beer, i.e. Day 0 sample, the average TPC measured was 240 mg/L. Upon dry-hopping at 19 °C and after just 24 h we observed a significant increase in TPC to 330 mg/L, representing a 38 % increase. The TPC increased further, to 49 % above starting levels after 3 days of dry-hopping, and then remained fairly constant for the rest of the dry-hopping period. Thus, the highest increase in total polyphenol content of the dry-hopped beers was observed after 3 days. The effect of dry-hopping temperature on total polyphenol content of dry-hopped beers is also evident in figure 6. Overall, there was proportionately less of an increase in TPC of the beers when dry-hopped at 4 °C, with a gradual increase in total polyphenol content over the dura-

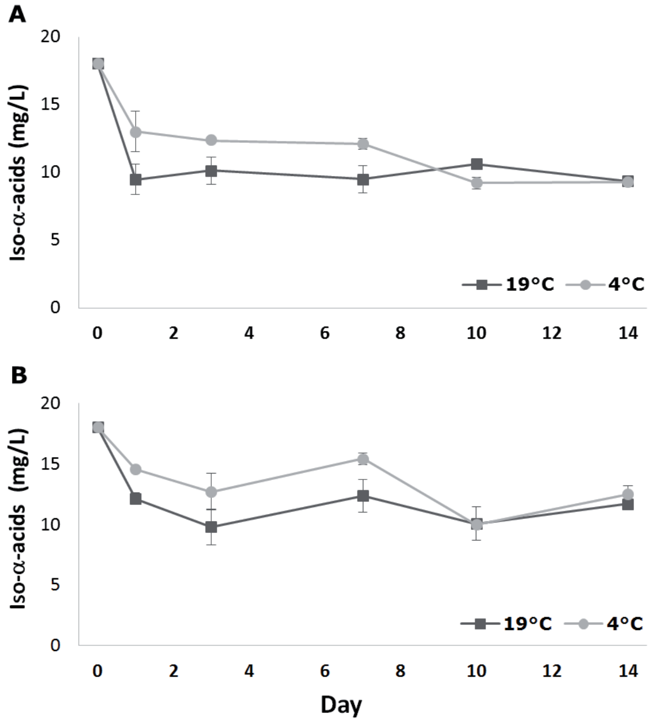

Fig. 4 **Changes in iso-α-acid concentrations over the course of dry-hopping at 4 and 19 °C with (A) high alpha Zeus and, (B) low alpha Hersbrucker**

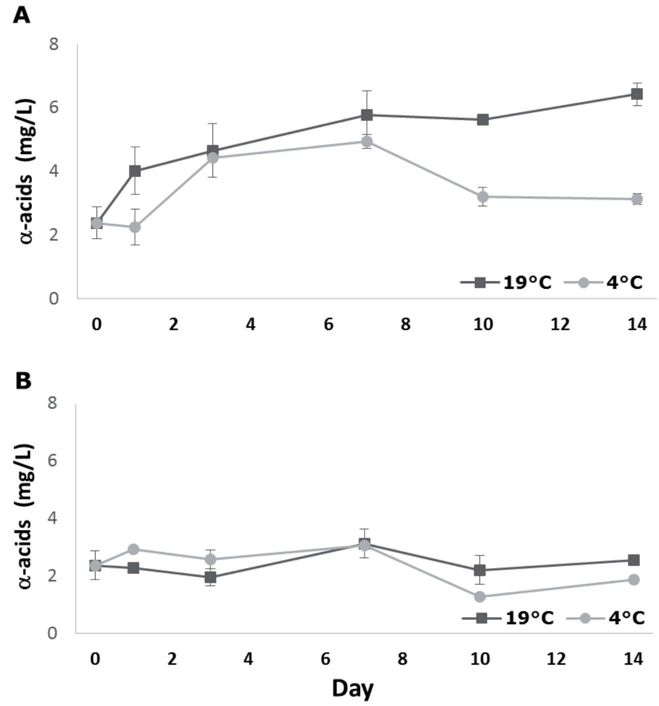

Fig. 5 **α-acid concentrations over the course of dry-hopping at 4 and 19 °C with (A) high alpha Zeus and, (B) low alpha Hersbrucker**

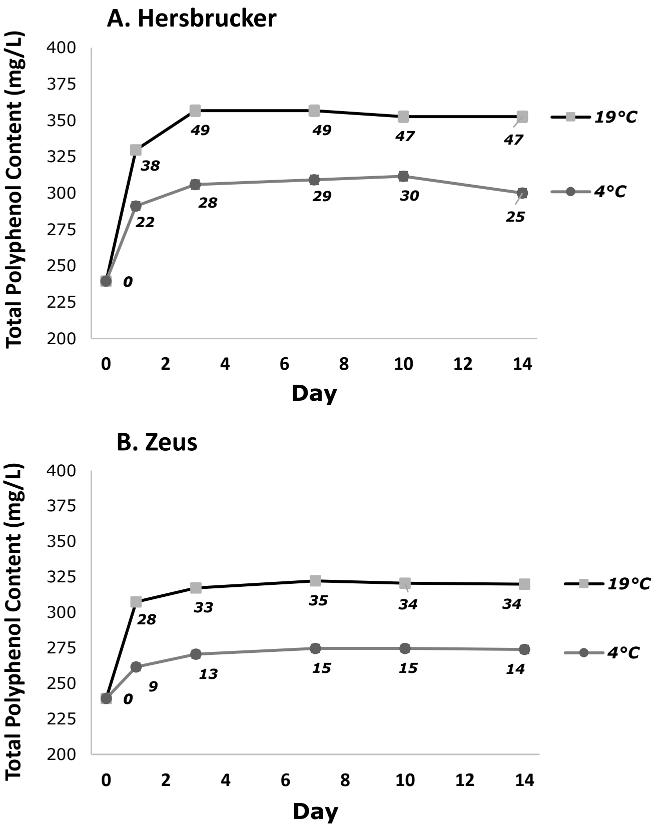

Fig. 6 **Increases in the total polyphenol content of beers dry-hopped with (A) Hersbrucker and (B) Zeus for 14 days at 4 and 19 °C. Data point labels show the relative percentage increase in total polyphenol content of beer during dry-hopping**

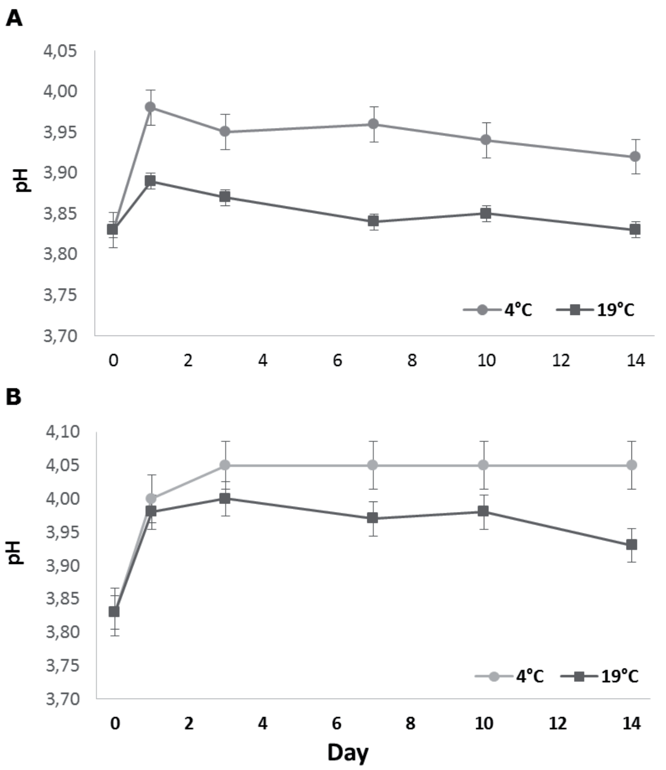

Fig. 7 **pH of dry-hopped beers at 4 and 19 °C for (A) Zeus and, (B) Hersbrucker**

tion of dry-hopping observed at this temperature, and reaching a peak at around day 10.

3.2.2 Zeus

For the beers dry-hopped with Zeus (Figure 6B), the biggest percentage increase in TPC occurred during the first 24 h of dry hopping at both temperatures investigated. However, the increase in total polyphenol content of the dry-hopped beers for Zeus was lower than that observed in Hersbrucker. Thus, the level of hop polyphenols extracted into beer during dry-hopping was clearly impacted by hop variety, but could also be controlled by dry-hopping at lower temperatures. To emphasise the impact of hop variety on the TPC of dry-hopped beers at 19 °C, our results show a maximum percentage increase of 34 % in total polyphenols in Zeus after 14 days of dry-hopping, which was lower than the increase observed in Hersbrucker after just 24 h of dry-hopping (38 %). A maximum increase of 15 % in total polyphenol content was observed when dry-hopping at 4 °C for the Zeus hop variety; this value was comparably higher at 30 % for the Hersbrucker hop variety.

3.3 Impact of dry-hopping on beer analytical parameters

3.3.1 pH

The measured pH of the dry-hopped beers over 14 days of dry-hopping is presented in figure 7. The results show, in agreement with a previous publication [14], an increase in beer pH during dry-hopping (Figure 7). Furthermore, our results show greater increase in beer pH at lower dry-hopping temperatures (4 °C) than at higher dry-hopping temperatures (19 °C); suggesting an impact of dry-hopping temperature on this parameter. The pH increase was observed for both hop varieties used in this study. This observation is of course important for the perception of bitterness in beer, since perceived bitterness intensity can be influenced by beer pH [14]. The increased beer pH may however be beneficial for the stability of iso-α-acids during storage, since these compounds are less prone to degradation at higher beer pH [9].

3.3.2 ABV (%)

A very interesting finding of this study relates to changes in beer alcohol content during dry-hopping. Over the course of dry-hopping, our results showed an increase in the alcohol content of the dry-hopped beers (Figure 8). This increase was greater (for both varieties) at the higher dry-hopping temperature investigated, i.e. at 19 °C. Our study found an increase of approximately 7 % in the alcohol content of beers dry-hopped at 19 °C. This finding has significant implications for craft brewers not just in terms of beer flavour, but also potentially in terms of their obligation to present accurate information about the alcohol contents of products. This sector has, in general, both a lower degree of control over packaged beer % ABV and a propensity to utilise dry hopping at very high dose rates.

3.3.3 Density

In addition to the observed increase in beer ABV (%), a concomitant reduction in beer density was observed over the course of dry-hopping (Figure 9). This reduction in density and increase in % ABV during dry-hopping was of greater magnitude at 19 °C, compared to 4 °C, for both hop varieties. In each case, there was a drop of approximately 0.5 % in beer density over the 14-day dry-hopping period. These findings indicate further attenuation of beer during the dry-hopping process, especially at higher dry-hopping temperatures, and suggest that further fermentation was initiated during the dry-hopping process. This may be due to the rousing process re-initiating fermentation of any available residual fermentable sugars in the beer, or the added dry hops serving as an additional source of sugars for yeast metabolism. Hops have been reported to contain approximately 2 % (w/w) monosaccharides [2], which could potentially be extracted into beer during dry-hopping (depending on alcohol content of beer and hop addition rate) and utilised by yeast for the production of more alcohol. Based on the monosaccharide content of hops, dry-hopping at a rate of 20 g/L could potentially add an additional 400 mg of sugar per litre of beer during the dry-hopping process. Recent reports have also suggested that this observation, termed 'hop-creep' by some brewers may be due to the effect of hop enzymes in breaking down non-fermentable sugars to fermentable sugars for the yeast to metabolise during dry-hopping [10]. However, further attenuation of beers during dry-hopping as a result of wild yeast (from added dry hops) cannot be totally discounted [5].

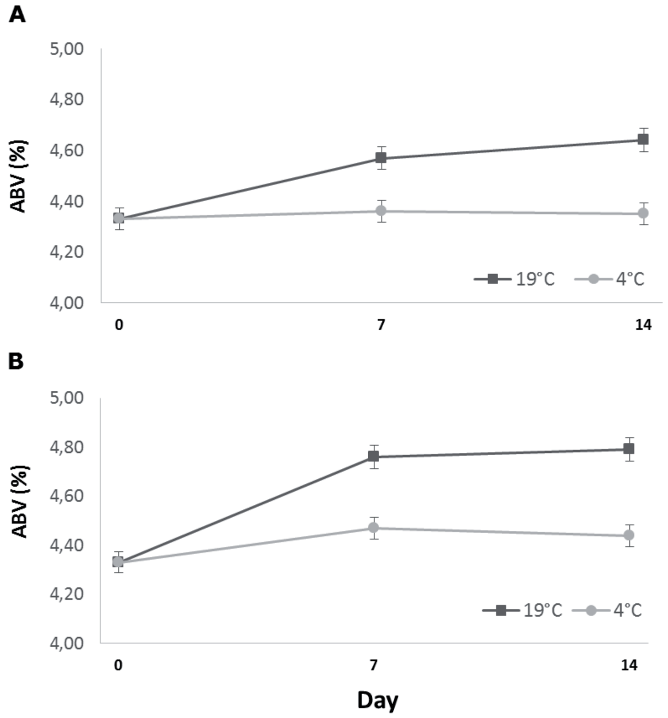

Fig. 8 Measured alcohol content of dry-hopped beers at 4 and 19 °C for (A) Zeus and, (B) Hersbrucker

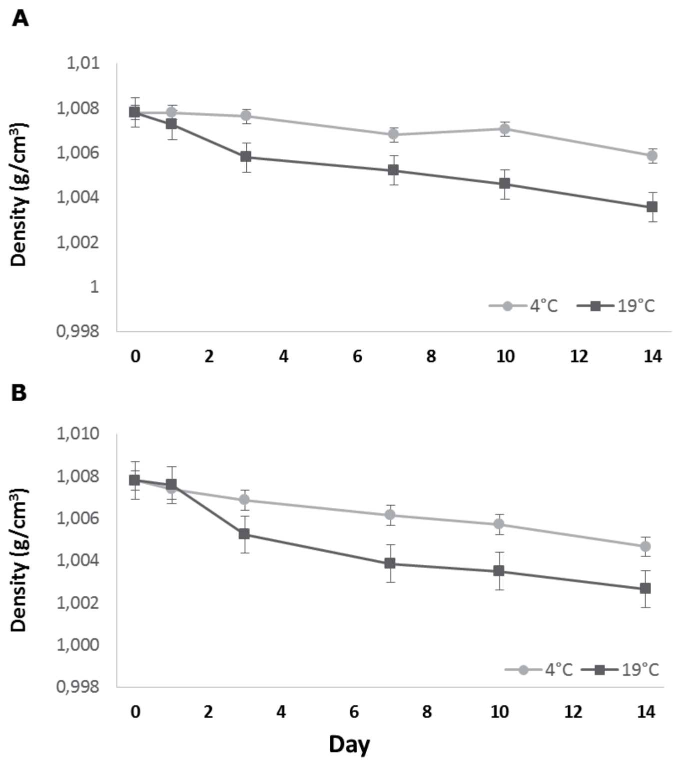

Fig. 9 Measured density of dry-hopped beers at 4 and 19 °C for (A) Zeus and, (B) Hersbrucker

3.4 Changes in the hop acid profile of spent hops

3.4.1 Losses of iso-α-acids

The observed reduction in concentration of beer iso-α-acids observed in this and other studies prompted us to investigate this matter further. The losses of these acids in the latter stages of beer production is mainly due to their adsorption onto the hops used for dry-hopping [13]. The results of this study in this regard are shown in figure 10 and confirmed that iso-α-acids are indeed adsorbed onto spent hop materials which brewers dispose of after dry-hopping. In figure 10A, the main iso-α-acid peaks separated by HPLC are shown as a reference. In figure 10B, the hop acids present in the Hersbrucker variety used for dry-hopping is presented, showing low levels of humulinones, α-acids and β-acids. In figure 10C, the hop acid composition of the spent hop slurry is presented, and immediately it can be observed (by simple retention time comparison) that iso-α-acids are present in the recovered spent hops. As shown in figure 10B, iso-α-acids were not present in the fresh hops used for dry-hopping, but after the dry-hopping process the spent hop slurry clearly illustrates the presence of iso-α-acid compounds. Furthermore, on closer inspection of the chromatogram of the spent hop, numerous oxidized hop acid compounds which are likely to be highly polar in nature were found to be present. It would be useful to identify some of these compounds with a view to better understanding their potential contribution to beer bitterness.

3.4.2 α-acids

The levels of α-acids remaining in the spent hops after dry-hopping was a further area addressed in this study. We found that approximately 15–25 % of the original -acid content remained in the spent hop after 14 days of dry-hopping under the conditions investigated, although this percentage can be expected to vary depending on the specific dry hopping conditions (temperature, duration, α-acid content, static/dynamic system etc.) adopted.

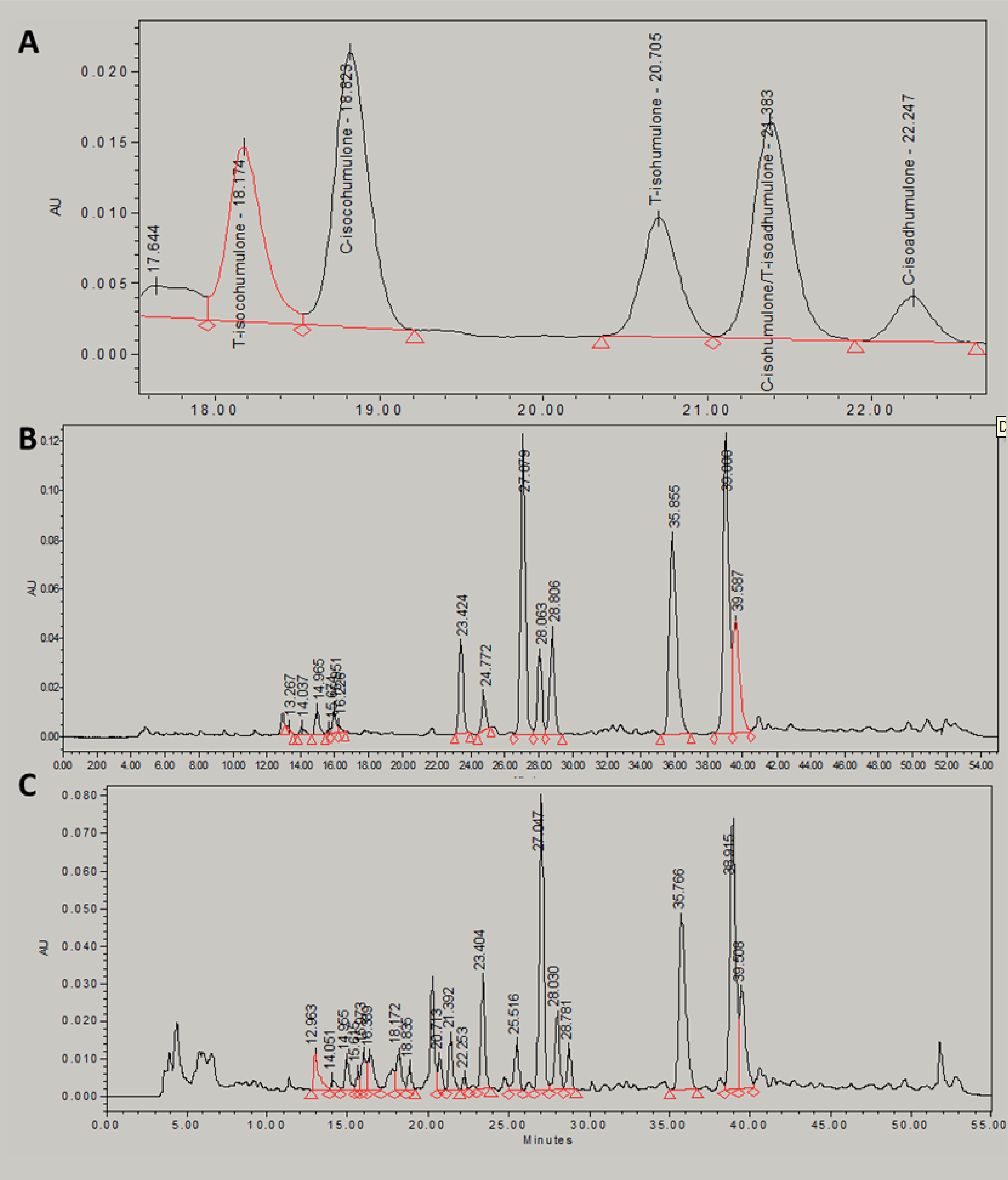

Fig. 10 HPLC separation of hop acids in fresh and spent hops after 14 days of dry-hopping at 4 °C. (A) Reference peaks for iso-α-acids: trans-isocohumulone (18.17), cis-isocohumulone (18.82), trans-isocohumulone (20.70), cis-isocohumulone & trans-isoadhumulone (21.38), cis-isoadhumulone (22.24). (B) Hop acid profile of fresh Hersbrucker; humulinones: cohumulinone (13.26), humulinone (14.96), adhumulinone (15.62); α-acids: cohumulone (23.42), prehumululone (24.77), humulone (27.07), postadhumulone (28.06) and adhumulone (28.80); β-acids: colupulone (35.85), lupulone (39.00) and adlupulone (39.58). (C) Hop acid profile of spent Hersbrucker hops, humulinones: cohumulinone (13.20), humulinone (14.95), adhumulinone (15.61); iso-α-acids: trans-isocohumulone (18.17), cis-isocohumulone (18.83), trans-isocohumulone (20.71), cis-isocohumulone & trans-isoadhumulone (21.39), cis-isoadhumulone (22.25); α-acids: cohumulone (23.40), prehumululone (24.72), humulone (27.04), postadhumulone (28.03) and adhumulone (28.78); β-acids: colupulone (35.76), lupulone (38.91) and adlupulone (39.50)

Table 1 **Total polyphenol content of fresh Hersbrucker and Zeus hops and their respective spent materials after 14 days of dynamic dry-hopping**

Hop variety	Hop condition	GAE (mg/L)	% loss
Zeus	Fresh	282	–
Hersbrucker	Fresh	252	–
Zeus @ 19 °C	Spent	143	49
Hersbrucker @ 19 °C	Spent	112	56
Zeus @ 4 °C	Spent	205	27
Hersbrucker @ 4 °C	Spent	162	36

3.5 Changes in total polyphenol content of spent hops

The TPC of fresh and spent Hersbrucker hops as determined by the Folin-Coilcateau assay is presented in table 1. The results show an average TPC of 282 and 252 mg GAE/L for fresh Zeus and Hersbrucker hop polyphenol extracts, respectively. In the spent hops recovered after dry-hopping at 19 °C for 14 days, the total polyphenol content remaining was 144 and 112 mg GAE/L of hop extracts, respectively. At this temperature, these numbers represent a reduction in TPC in the spent hops of 49 and 56 % in the spent Zeus and Hersbrucker hops, respectively. After dry-hopping at 4 °C for 14 days, the total polyphenol content of spent Zeus and Hersbrucker was 205 and 162 mg GAE/L of hop extracts, representing a reduction in total polyphenol content of 27 and 36 %, respectively. These lower values at the lower dry-hopping temperature (4 °C) mean that less polyphenols were extracted out of hops into beer at this temperature relative to 19 °C. This further emphasises the significant effect of temperature on the dynamic extraction of hop polyphenols and related compounds into beer as discussed in Section 3.2. Greater reductions in TPC of the spent hops were observed at 19 °C than at 4 °C for both hops. Furthermore, these results also confirm greater extraction of polyphenols into beer during dry-hopping from the Hersbrucker hops than from Zeus, as was already identified from the beers themselves in Section 3.2. Accordingly, approximately 50 % of the original content of hop polyphenols remains in the spent hops that brewers currently dispose of as waste. The polyphenol and residual hop acid contents of spent hop slurries indicate that the slurries could be an added-value material for brewers to consider, for example, by re-introducing good quality spent hops back into the kettle for part of the bittering of a fresh batch of wort.

4 Conclusions

This study investigated the impacts of dry-hopping at 4 and 19 °C, using a low alpha (Hersbrucker) and high alpha (Zeus) hop variety, on the resulting profiles of several hop acid compounds over a 14-day period. Our results suggest that the concentration of bitter tasting humulinones in dry-hopped beers is not solely down to the extraction of these compounds from hops during the dry-hopping process, and that the formation of these compounds from (α-acids) can occur during dry-hopping. Humulinone concentrations during dry-hopping were significantly impacted by hop variety and temperature; dry-hopping with high alpha hops at warmer temperatures (19 °C) resulted in higher concentrations of these compounds compared to dry-hopping at 4 °C with a low alpha hop. Humulinones are worthy of greater attention from craft brewers who wish to better understand both the analytical and sensory bitterness of their products, due to their bitterness intensity and their high concentrations in dry-hopped beers.

We also provide conclusive evidence to show that losses of iso-α-acids through dry hopping occurred due to their adsorption onto spent hop materials. The greatest drop in the concentration of iso-α-acids was observed after 24 h of dry-hopping at both temperatures investigated. A significant increase in α-acid concentrations during dry-hopping was only observed in beers dry-hopped with high alpha Zeus at the higher dry-hopping temperature of 19 °C. The total polyphenol content of dry-hopped beers increased significantly after just 24 h of dry-hopping for both hop varieties and at both temperatures investigated; with the rate of polyphenol extraction found to be significantly dependent on dry-hopping temperature and hop variety. There was greater extraction of polyphenols into beer at 19 °C than at 4 °C in both hop varieties, although at both temperatures, more polyphenols were extracted into the dry-hopped beer from Hersbrucker hop than from Zeus. This is logical, as low alpha hop varieties usually contain more polyphenols than high alpha hop varieties [17]. Significantly, dry-hopping also had an impact on key beer analytical parameters. In summary, we observed an increase in beer pH, a decrease in beer density and increase in alcohol content over the course of dry-hopping with both hop varieties. The effects on %ABV and density were greater at the higher dry-hopping temperature of 19 °C; we conclude that the presence of suspended yeast in beer during dry-hopping may well have initiated further fermentation during this process – with sugars extracted from the dry hops into beer during dry-hopping further promoting yeast metabolism. Consequently, the presence of suspended yeast in beer during dry-hopping may have further significant impacts on overall beer flavour beyond those already observed in relation to volatile hop compounds. Alternatively, our observation in relation to further attenuation during dry-hopping may be due to the activity of wild yeast (from the dry hops) introduced during dry-hopping [5]. Brewers dry-hopping beer with some yeast in suspension should endeavour to measure beer parameters (pH, ABV etc.) after dry-hopping.

The results presented on spent hops provide some basis for brewers to consider utilising spent hops in the brewhouse as a sustainable approach towards reducing waste in the brewing process, and as a potential solution to the shortage of hops created by high demand. Dry hopping remains an exciting process which offers brewers opportunities to experiment and stand out by creating unique beers. Continued research into this topic will no doubt further our knowledge of this complex, yet important process for beer production.

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