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Nitrogen fertility practices in the field influence the accumulation of nitrate during the production of hop-forward beer

Hops and malt are the two main sources of nitrate in beer, with hops being the primary source in beers that are late-hopped or dry-hopped. A proof-of-concept study was performed to characterize how nitrogen fertilizer dose applied in the field during hop production influences nitrate accumulation in hop-forward beers. To quantify the dose-response relationship two contrasting nitrogen fertilizer rates, 90 and 269 kg/ha, were used to produce hops that differed in their nitrate concentrations (852 and 2651 mg/kg of nitrate, respectively). These rates were selected to represent extremely low and extremely high nitrogen fertilizer commercial rates, respectively. Hop-forward beers (kettle hopped at 0.52 g/L, whirlpool hopped at 2 g/L, and dry-hopped at 4 g/L) were produced with these hops and nitrate accumulation was quantified after each hop technique to determine the overall contribution of each hopping technique on the nitrate concentration in beer, as well as if the different hop treatments yielded beers with different nitrate levels. Nitrate accumulation following each hop technique was associated with nitrate concentration of the hops and, ultimately, the rate of nitrogen fertilizer applied in the field. As supported by previous studies, nitrate accumulated quantitatively with respect to the different hop techniques, with dry-hopping resulting in the largest increase in nitrate, followed by whirlpool additions, and then kettle hop additions. Although beer is a minor dietary source of nitrate, our results indicate that reducing nitrogen fertilization rates in the field can reduce nitrates in hop-forward beers.

Descriptors: brewing, dry-hopping, fertilizer, hop-forward beer, nitrate accumulation, nitrogen

1 Introduction

Driven in large part by the craft industry, the growth of hop-forward beer styles has increased substantially [1]. Dry-hopping, which has been defined as the extraction of volatile and non-volatile components out of hops into fully or partially fermented beer, is one of the main techniques brewers use to add hop aroma and flavor to these styles [2, 3]. Historically, dry-hopping was a technique use to improve the shelf life of beer. However, contemporary craft brewers use dry-hopping to impart more intense or unique aromas to beer without imparting substantial bitterness.

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Dry-hopping, which is performed on cool fermenting wort or cold beer, allows hop essential oils to be extracted at higher levels in comparison to both kettle and late hopping. Due to the high quantities of hops used for dry-hopping in hop-forward beer styles, extraction of unwanted substances such as nitrate have also become a consideration [4]. Previous research has shown that hops are one of the main sources of nitrate in beer [5]. Nitrate is a naturally occurring ion in living organisms and due to its high water solubility nitrate is extracted out of hops during dry-hopping at high rates (>75 %), potentially resulting in relatively high concentrations in beer depending on several factors [6–9]. Although it is dependent on the hop variety and beer style, concentrations of nitrates in hops have been reported to range ~300 – 10 000 mg/kg [7, 10], while nitrate concentrations in beer have been shown to range from 5 – 40 mg/L [6, 8].

While nitrate is naturally occurring in humans and not very toxic, drinking water guidelines and standards exist for nitrate primarily because of the potential sensitivity of bottle-fed infants [11]. The overall health impacts of nitrate for adults is unresolved and nitrate consumption may even be considered beneficial in some contexts [12–14]. Approximately 80 % of dietary nitrate is derived from vegetables, with fruit and processed meats being secondary sources [15]. Although beer is an overall minor dietary source of nitrate and the health consequences of nitrate consumed in beer has not been evaluated, there are motivations to understand determinants of nitrate accumulation in beer and how to mitigate such accumulation.

Table 1 Hop cone quality specifications and yield of Tomahawk hops produced in 2015 using two doses of nitrogen fertilizer

Treatment	Nitrogen rate (kg/ha)	Dry matter (%)	Yield (kg/string)	Essential oil (ml/100g)	α-acids (%)	β-acids (%)	Nitrate[a] (mg/kg)
Low N	90	26.4	0.61	4.1	19.7	5.7	852
High N	269	26.0	0.66	3.8	18.6	5.6	2651

[a] Nitrate concentration was determined by extraction from ground cones using KCl and assayed using a Lachat Autoanalyzer following a cadmium reduction flow injection protocol [10]

Nitrogen fertility practices during hop production can influence both nitrate levels and multiple brewing quality factors in hops, as well as certain diseases and arthropod pests in the field [10, 16–18]. In hop production regions in the western United States, recommended application rates of nitrogen range from ~112–225 kg N/ha depending on variety, age and expected yield [10]. Recent research indicated that applying the lowest feasible nitrogen rate and ceasing nitrogen applications by bloom could optimize certain cone quality factors while minimizing nitrate accumulation in hop cones [10]. However, the fate of nitrate and its accumulation during the brewing process when brewing with these cones was not assessed explicitly in that study. Therefore, the objective of this study was to assess the impact of nitrate accumulation during the production of a hop-forward beer and assess the contribution of different hopping techniques to the total nitrate concentration in beer.

2 Materials and Methods

2.1 Hop cones from nitrogen fertility trials

Nitrogen fertilizer dose-response trials were conducted in Washington State during 2015 in a commercial yard of cultivar Tomahawk, as described in detail in *Iskra,* et al. [10]. In brief, the hop cones used in the current study were harvested from a hop yard that was planted in 2008 on a 4.3 m wide row spacing with 1 m between each hill (plant) under a standard 5.5 m trellis. Thirty-four kilograms per ha of ammonium nitrate was banded in late winter across the whole field. The yard was irrigated by surface drip irrigation and additional nitrogen required to deliver 90 kg/ha and 269 kg/ha respectively, was injected through the drip on bi-weekly intervals as urea ammonium nitrate (32-0-0) or calcium ammonium nitrate (21-0-0). Each nitrogen rate treatment was replicated four times in a randomized complete block design. We refer to these nitrogen rates as low and high for brevity. We emphasize that the two nitrogen fertilizer rates were selected for demonstration of a dose-response; neither rate should be considered optimum for all production situations [10].

After harvest, hop cones from each replicate plot were analyzed for moisture content, percentage of α- and β-acids, hop storage index (HSI), and total essential oil content using the ASBC standard methods [19]. Nitrate was extracted from ground hops in a KCl solution and assayed colorimetrically using a Lachat Autoanalyzer (Hach, Loveland, Colorado, USA) following a cadmium reduction flow injection protocol [10]. In brief, samples were dried to completion, ground to a fine powder, and then covered with 30 mL of a 2 M KCL solution. After shaking for 30 min at 250 rpm, the samples were filtered using a Whatman #1 filter and the filtrate was analyzed for nitrate concentration.

In order to obtain enough hops to brew with, cones from the four field replicates were mixed into one composite sample for each of the low and high nitrogen treatments. Mean chemical values for these samples were used for subsequent brewing calculations, as described below (Table 1).

2.2 Pilot batch brewing trials and sample collection

Two hop-forward beers were made on a pilot scale (~2.5 hL) using hops from each of the low and high nitrogen fertilizer doses. Wort (~14.8 °P original gravity) was made from a grist of 100 % pale ale malt (Great Western Malting, Vancouver, Washington, USA), utilizing a single infusion mash (68.8 °C). The mineral content of the brewing water was adjusted using 18.0 mg/L of $CaCl_2$ and 38 mg/L of $CaSO_4$.

Three hopping techniques were performed during the brewing/fermenting process: beginning of boil, late-hopped in the whirlpool, and dry-hopped post fermentation and clarification. Kettle hopping at 0.52 g/L was performed at the beginning of a 60-minute boil to achieve 35 mg/L of iso-α-acid based on the hops' α-acids concentration. Late hopping was performed at 2 g/L and dry-hopping was performed at 4 g/L. These rates were chosen because at the time of the experiment they represented typical commercial hopping rates used in the U.S. The wort was fermented at 20 °C for 8 days with an ale yeast (*Saccharomyces cerevisiae*, Wyeast 1056) pitched to achieve 3.64×10^{12} cells. Following diacetyl reduction, the beer was cooled to 15.5 °C and the flocculated yeast was removed by purging the fermenter cone prior to dry-hopping.

The green beer was then dry-hopped by adding coarsely ground hops to the fermentation vessels and held at 15.5 °C for 24 h. After this initial 24 h, the temperature was dropped to 1.1 °C and the beer held for another 24 h. The green beer was then filtered to stop the dry-hopping process using a plate and frame filter with cellulose pads (HS2000, Pall Corporation, Port Washington, New York, USA). Dissolved oxygen (DO) was monitored during filtration using an Orbisphere 3100 Portable Oxygen Analyzer (Hach, Loveland, Colorado, USA). Bright beer was not collected until the DO was below 80 mg/L. After the DO was within specification, bright, filtered beer was collected in a closed 19.6 L stainless steel keg with enough backpressure to reduce foaming. Between each filter run of the two batches of beer, filter pads were exchanged to prevent product carry-over between treatments. Filtered beer was stored at 2 °C and under CO_2 overpressure (83 kPa). Throughout the different stages of the brewing process two 250 mL samples were collected and frozen until analysis. The final beer specifications (ethanol, residual extract, original gravity and pH; Table 2) were measured using an Anton-Paar Alcolyser with supporting pH module (Anton Paar USA, Ashland, Virginia, USA) using ASBC standard methods.

Table 2 **Quality specifications of beer produced with hops receiving a low or high rate of nitrogen fertilizer**

Treatment	Alcohol (% v/v)	Real extract (% w/w)	pH
Low N	6.85	5.35	4.68
High N	6.54	5.50	4.67

2.3 Accumulation of nitrate throughout the brewing process

Prior to nitrate measurement, the samples were thawed, warmed to room temperature (~20 °C), and vortexed. Nitrate measurements were made with an VWR symphony B30 PCI three channel benchtop meter (VWR, Visalia, California, USA) and an Intellical™ ISENO3181 Nitrate (NO_3^-) Ion Selective Electrode (ISE) probe (Hach). The probe was calibrated with 1, 10, 100, and 1,000 mg/L standard solutions. Prior to calibration and measurement, a nitrate ionic strength adjustor powder pillow was added and allowed to dissolve in 25 mL of the standards and samples. Nitrate standard solutions and the ionic strength adjustor powder pillow were sourced from Hach. Each of the treatments was measured in triplicate unless otherwise noted.

3 Results and Discussion

3.1 Accumulation of nitrate throughout the brewing process

Nitrate accumulation was tracked throughout the production of hop forward beers made solely with either hop cones from the high or low nitrogen fertilizer dose treatments (Fig. 1). As supported by previous research [5–7], hop additions were the primary source of nitrate during the brewing process. Dry-hopping resulted in the largest increase in nitrate (~16.7 and ~30.5 mg/L, for the low and high fertility rates, respectively), followed by whirlpool hop additions (~1.9 and ~5.5 mg/L), and lastly the kettle hop additions (~0.83 and ~2.3 mg/L). Nitrate concentration in the beer depended on the nitrogen dose applied to the hop plants during the production of the hops. The field treatment that was fertilized with the low nitrogen dose produced hops with about half the nitrate concentrations as the hops that received the higher nitrogen dose (Table 1), and subsequently contributed half as much nitrogen in the brewing process. The dry-hopping approach used in this study involved waiting for the fermentations to attenuate fully and removing yeast from the fermenter prior to dry-hopping. It should be noted that many brewers perform dry-hopping by adding hops during active fermentation when yeast cell metabolism is high and yeast are still dividing. This distinction is potentially important because some yeast, notably *Brettanomyces spp.*, are able to assimilate nitrate [8, 9]. Therefore, the values in the present study could overestimate what might occur in a commercially-produced IPA depending on the yeast strain used and the dry-hopping approach.

Nevertheless, these results directly demonstrate that using the lowest feasible nitrogen rate during the production of hops could be an effective strategy to reduce nitrate concentration in hop-forward beer. Crop management therefore can complement other techniques that have been proposed previously [5], such as using supercritical CO_2 hop extracts or concentrated lupulin powders, and possibly selected yeast strains to reduce beer nitrate concentrations without sacrificing beer quality. While we focused on nitrogen dose in the present study, other hop production practices that reduce nitrate accumulation in hops are similarly expected to reduce nitrate concentration in beer [16, 20]. Given the importance of hop quality in producing hop-forward beers, reducing nitrogen fertilizer dose as much as feasible during hop production not only moderates nitrate accumulation in the final beer but can also maximize quality determinants of principial concern to brewers [10]. For example, higher nitrogen fertilization rates were found to yield hops (i.e. Tomahawk and Willamette) with lower α-acids and essential oil concentration.

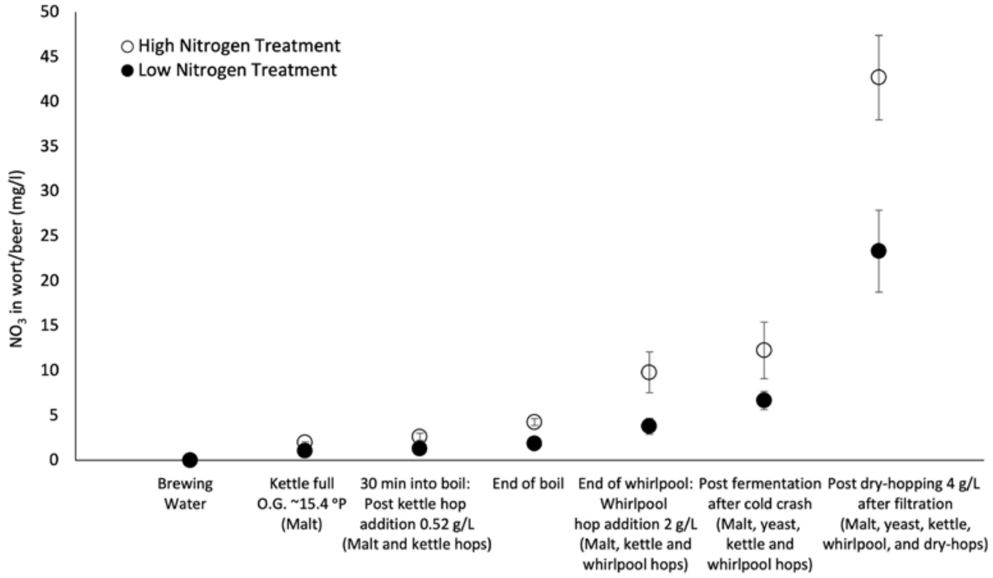

Fig. 1 **Nitrate accumulation throughout brewing hop-forward beers made from hops that received either a low or high rate of nitrogen fertilizer. Nitrate concentration was measured using an Intellical™ ISENO3181 Nitrate Ion Selective Electrode probe. Error bars represent twice the standard error of the measurement**

4 Conclusions

Nitrate accumulation in beer was commensurate with hop technique, with the largest increase associated with dry-hopping; whirlpool and kettle additions had relatively lesser effects with the hopping rates examined herein. The total nitrogen fertilizer dose applied to the hop plants in the field also clearly influenced nitrate accumulation following each hop technique and the overall nitrate concentration in the finished beer. This study demonstrates conclusively that hop production factors in the field can influence nitrate accumulation throughout the brewing process. Given the importance of hop quality in hop-forward beers, reducing nitrogen fertilizer dose as much as feasible during hop production not only moderates nitrate accumulation in brewing but as shown in other work can also maximize quality determinants of principial concern to brewers such as α-acids and essential oil content [10]. Additional studies are needed to evaluate the impact of nitrogen fertilization rates on the nitrate accumulation in other hop varieties as well as in different growing regions.

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