Phytochemical Characterization of Native New Mexico Hops

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SUMMARY. Neomexicanus hops (*Humulus lupulus* var. *neomexicanus*) are receiving increased attention within the craft beer and nutraceutical industries. Characterization of bittering acids and essential oils in two neomexicanus varieties revealed wide ranges of bittering acid compositions and distinct essential oil profiles compared with ‘Cascade’ common hops (*H. lupulus*). Total phenolic content (TPC), expressed as gallic acid equivalent (GAE), in neomexicanus hops ranged from 50 to 100 mg·g⁻¹ GAE, consistently higher than published literature values for hop TPC (2 to 50 mg·g⁻¹ GAE). Results indicate that, compared with ‘Cascade’, neomexicanus hops have unique phytochemical characteristics, which may lead to new applications in brewing and nutraceutical fields.

Common hops (*Humulus lupulus*) used in brewing are typically of European genetics and cultivated between latitudes 45°N to 50°N (Dodds, 2017). Lesser studied, neomexicanus hops (*H. lupulus* var. *neomexicanus*) are native to the western United States, predominantly in the Rocky Mountain region between latitudes 32°N and 42°N (Smith et al., 2006). Neomexicanus hops are gaining interest among craft brewers and hop breeders (Bannerman, 2019; Jones, 2018). Named neomexicanus hop varieties and their

hybrids include Amalia, Latir, Medusa (or Multihead), Neo-1, Sabro, and Zappa (CLS Farms, n.d.; Jones, 2018; Santa Fe Brewing Co., n.d.). Phytochemicals of interest include bittering acids and essential oils for their brewing properties as well as polyphenols, particularly xanthohumol, for nutraceutical properties. Study objectives were to survey neomexicanus hop cone chemistry and compare results to the chemistry of ‘Cascade’ common hops.

Materials and methods

PLANT MATERIAL. ‘Multi-head’ and ‘Neo-1’ neomexicanus hops and ‘Cascade’ common hops were cultivated under identical conditions at New Mexico State University Agricultural Science Center at Farmington (lat. 36.68915°N, long. 108.31277°W, elevation 1720 m). Hops were harvested in stages when cones felt “dry and papery” and lupulin glands were dull yellow. Cones were air dried (48 h) and stored in vacuum-sealed bags in darkness at 0 °C. Samples were ground using

a food chopper (Homeleader K56-016; Cixi, Zhejiang, China) before analysis.

ESSENTIAL OIL ANALYSIS. Essential oil analysis was performed according to American Society of Brewing Chemists (ASBC, St. Paul, MN) methodology (ASBC, 2008a). Ground hops (100 g) were distilled in 3 L of water for 4 h, and the oils were collected in a calibrated graduated receiver. Oils were transferred into amber vials and stored in darkness at 0 °C until gas chromatography–mass spectrometry (GC-MS) characterization.

BITTERING ACID ANALYSIS. Analysis of alpha and beta acid profiles followed ASBC (2008b) methodology. A mixture of ground hops (10 g), 20 mL of high-performance liquid chromatography (HPLC)-grade methanol, and 100 mL of diethyl ether was shaken for 30 min; followed by the addition of 40 mL of 0.1 M hydrochloric acid and continued shaking for 15 min. The mixture was gravity separated. Supernatant (5 mL) was collected and added to a 50-mL volumetric flask and brought to volume with HPLC-grade methanol. The solution was vacuum filtered through a Buchner funnel with filter paper (Grade 4; Whatman International, Maidstone, England) and transferred to an amber bottle. Quantification was performed immediately by HPLC using International Calibration Extract 4, obtained from ASBC, as an internal standard.

POLYPHENOL ANALYSIS. An extract was prepared from 10 g of hops in 250 mL of 45% ethanol (Xiong et al., 2006) and incubated in a water bath at 60 °C for 90 min (Kowalczyk et al., 2013). Samples were centrifuged after incubation. Liquid fractions were collected, vacuum filtered through a Buchner funnel lined with filter paper (Whatman Grade 4), and stored in amber bottles in darkness at 0 °C. Total phenolic content (TPC) of hops extracts was determined by the Folin–Ciocalteu

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
10	%	mg·g ⁻¹	0.1
29,574	fl oz	μL	3.3814 × 10 ⁻⁵
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
3.7854	gal	L	0.2642
25.4	inch(es)	mm	0.0394
28.3495	oz	g	0.0353
(°F–32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Table 1. Composition of major bittering acid components, total essential oils, and total phenolic content in New Mexico-grown hops.

Analysis	‘Cascade’ common hops	‘Neo-1’ neomexicanus hops (mean ± SD)	‘Multi-head’ neomexicanus hops
Bittering acid components (%) ^z			
cohumulone	12.06 ± 0.29 a ^y	16.08 ± 4.86 a	3.47 ± 0.64 b
n+adcohumulone	30.39 ± 0.92 a	16.68 ± 4.06 b	3.89 ± 0.59 c
colupulone	14.54 ± 0.16 a	11.45 ± 3.43 a	17.72 ± 2.70 a
n+adlupulone	17.97 ± 0.15 a	4.46 ± 1.15 c	8.63 ± 1.64 b
Total alpha-acids	42.44	32.76	7.35
Total beta-acids	32.51	15.91	26.36
Total essential oils (mL) ^{x,w}	0.75	0.15	0.15
Total phenolic content (mg·g ⁻¹ gallic acid equivalents) ^{z,w}	50.08 ± 1.59 a	94.29 ± 6.05 b	92.41 ± 5.71 b

^zn = 3.

^yAt $P \leq 0.05$, means in the same row with the same letter are not significantly different from each other per Tukey’s honestly significant difference post-hoc test.

^xn = 2.

^w1 mL = 0.0338 fl oz, 1 mg·g⁻¹ = 0.1%.

Fig. 1. Qualitative comparison of gas chromatography–mass spectroscopy chromatograms (not to scale) for New Mexico-grown hops: (A) ‘Cascade’ common hops, (B) ‘Multi-head’ neomexicanus hops, and (C) ‘Neo-1’ neomexicanus hops.

method, with modifications (Singleton and Rossi, 1965): 100 μ L of extract in 900 μ L deionized water (dH₂O) was added to 4 mL of dilute (1:5 dH₂O) Folin–Ciocalteu’s phenol reagent (Sigma-Aldrich, St. Louis, MO). The mixture was vortexed and left to stand for 3 min; then 5 mL of 7.5% sodium carbonate was added. This mixture was vortexed again, then incubated at room temperature in darkness for 60 min. Absorbance was measured after incubation at 740 nm in a spectrophotometer (SpectraMax 384; Molecular Devices, San Jose, CA) and converted to GAE concentration using a standardization curve ($r^2 = 0.998$) of gallic acid solutions prepared the same way.

STATISTICAL ANALYSIS. Three replicates were performed for bittering acid and polyphenol analyses. Due to limited biomass material, only two replicates were obtained for essential oil distillation. Statistical analysis was performed using SAS (version 9.4; SAS Institute, Cary, NC). Results were evaluated for statistical significance at $\alpha = 0.05$ using a two-way analysis of variance with Tukey’s honestly significant difference post-hoc test.

Results

Compared with ‘Cascade’, neomexicanus hops grown under the same conditions had consistently

higher TPC and lower essential oil yields (Table 1). Additionally neomexicanus essential oil profiles varied distinctly from ‘Cascade’. Visual comparison of essential oil GC-MS chromatograms (Fig. 1) qualitatively indicates the presence of compounds in neomexicanus that are not present in ‘Cascade’. Table 1 illustrates that neomexicanus cannot be entirely categorized as a bittering hop. ‘Neo-1’ has high alpha-acids, while ‘Multi-head’ has high beta-acids. Here, ‘Cascade’ has an alpha:beta ratio of 1.3, which falls within the range of reported ratios (0.64 to 1.55) for the variety (Gooding Farms, n.d.).

Discussion

During centrifugation of hops: ethanol mixtures for polyphenol extraction, seeds were discovered. Low total essential oil content in neomexicanus varieties can be attributed to seed presence. Both neomexicanus varieties showed high TPC compared with ‘Cascade’ common hops, which had no seeds (Table 1).

The 2018 growing season received 126 mm of precipitation (79 mm below average), endured average temperatures of 47 °F in January and 90 °F in June (6 and 3 °F above average, respectively) (O’Neill et al., 2018), and experienced spider mite infestation. Hops are generally dioecious, with female plants selected for cone production. However, these environmental stressors likely triggered a hermaphroditic response resulting in seed formation

(Sirrione, 2017). The relationship between drought, seed presence, and high TPC is unclear; polyphenol content, however, appears to be less related to drought (Čeh et al., 2007).

Conclusions

‘Multi-head’ and ‘Neo-1’ neomexicanus hops had significantly higher TPC than published literature values for hops extracts from traditional growing regions. This supports the potential for neomexicanus hops to be a good source of xanthohumol (a subject of ongoing work). The high TPC of all the New Mexico-grown hops suggests other environmental components influencing phenolic content. If increased secondary metabolite production results from environmental factors, then this could possibly be a general characteristic of neomexicanus hops that developed under environmental stressors of New Mexico and the surrounding southwestern U.S. region (drought, high elevation, and increased solar irradiation). Data evaluated herein add to potential alternative uses for neomexicanus: as an antimicrobial agent or source for natural polyphenols. While neomexicanus hops have potential as an aroma hop, further breeding experiments to minimize hermaphroditism are needed. Neomexicanus hops examined here are unlikely to serve as a bittering hop; the high cohumulone fraction in ‘Neo-1’ would impart an undesired flavor,

and the low fraction of alpha acids in ‘Multi-head’ make it a poor bittering candidate.

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