Zachary Hutzell, Samuel O. Ogundipe, Donglin Zhang and Joon Hyuk Suh

UHPLC quantification of α- and β-acids in Georgia-grown hops

To support the emerging hop (*Humulus lupulus* L.) industry in the southeastern United States, a reversed-phase ultra-high performance liquid chromatography (UHPLC) method was optimized and validated to quantify α- and β-acids in 11 Georgia-grown cultivars. The method, utilizing an ICE-4 standard on a C18 column, demonstrated good selectivity, sensitivity, linearity ($R^2 \geq 0.999$), intra- and interday precision (%RSD ≤ 3.24%) and accuracy (≥ 94.6%), indicating consistent quantification across major α and β acid constituents. Analysis was conducted on a single harvest from the 2025 growing season. Quantification of Cascade, Cashmere, Centennial, Chinook, Cluster, Columbus, Comet, Fuggle, Magnum, Nugget, and Zeus revealed significant cultivar-dependent responses under Georgia field conditions. While total α-acid concentrations in traditionally high alpha cultivars such as Zeus (118.06 $mg \cdot g^{-1}$) and Columbus (86.08 $mg \cdot g^{-1}$) were lower than Pacific Northwest standards, Comet (93.74 $mg \cdot g^{-1}$) and Cascade (73.95 $mg \cdot g^{-1}$) achieved concentrations comparable to commercial ranges. These results suggest that environmental conditions in the southeastern United States influence bitter acid accumulation; however, factors such as pathogen pressure, daily light integral, and other agronomic variables may also contribute, requiring further multi-season evaluation. Certain cultivars nonetheless show potential to produce cones of commercial bittering quality under regional conditions. Although oil composition and aroma quality remain to be analyzed for brewers, reporting the acid content is a fundamental first step in assessing the biosynthetic potential of these plants in Georgia's environment.

Descriptors: *Humulus lupulus*; chemical analysis; heat stress; phytochemical profiling; secondary metabolites

1 Introduction

Common hops, *Humulus lupulus* L., are a major ingredient in beer, along with water, malt, and yeast. Hops are valued for their contribution to bitterness, flavor, foam stability, and antimicrobial activities in beer [1]. Brewers primarily evaluate two major quality parameters when selecting hops: alpha acids and aromatic essential oils. The major alpha acids present in hops are n-humulone, cohumulone, and adhumulone. When boiled, these compounds are isomerized, producing iso-alpha acids, which are responsible for imparting distinct bitter flavor to the final beer. The beta acids in hops are mainly comprised of n-lupulone, colupulone, and adlupulone. In hop storage prior to brewing, and to some extent during the boil, these compounds are oxidized (rather than isomerized), forming hulupones [2,3,4]. However, because beta acids possess poor solubility in wort, their oxidation products are typically found in negligible amounts in finished beer. Consequently, alpha acid concentration remains the primary metric brewers use to determine a hop's bittering potential. Equally critical to brewer selection is the hop aroma profile. Essential oil compounds such as hydrocarbon terpenes (i.e. myrcene & humulene) confer aromatic flavor notes that define many modern beer styles, serving as a primary decision criterion for brewers.

The Woody Ornamentals Lab at the University of Georgia has grown 11 hop cultivars to evaluate the feasibility of hop production in Georgia. The cultivars selected are Cascade, Cashmere, Centennial, Chinook, Cluster, Columbus, Comet, Fuggle, Magnum, Nugget, and Zeus. When grown in the Pacific Northwest (PNW), these cultivars present quantifiable and predictable concentrations of α- and β-acids. However, the chemical profile of specific cultivars, such as Comet, has been shown to fluctuate significantly based on regional environmental influences [5]. Additionally, Cascade hops grown in Florida contained a lower concentration of beta acids than those grown in the PNW [6]. Climatic and environmental factors affect the accumulation of α- and β-acids [7], thereby posing a unique opportunity for Georgia hop growers; hop cultivars with identical genetics to classically grown PNW hops may present novel flavors when grown in Georgia.

Expanding hop production into southern regions such as Georgia presents unique physiological challenges due to the plant's pho-

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toperiodic sensitivity [6]. Hops are long-day plants that require a specific "critical day length" to remain in a vegetative state and accumulate sufficient biomass before transitioning to flowering [8]. In traditional growing regions such as the PNW, summer day lengths typically exceed 15-16 hours. However, at southerly latitudes like the Georgia Piedmont (approx. 34° N), the maximum natural day length at the summer solstice is approximately 14.4 hours. This shorter photoperiod is often below the critical threshold for many high-alpha cultivars, causing them to perceive "short days" prematurely. This results in early floral initiation and stunted vertical growth, which significantly limits cone yield and quality. While supplemental lighting is a proven strategy to mitigate these photoperiodic limitations in low-latitude environments [6], this study deliberately utilized natural photoperiods to evaluate the inherent physiological performance of the 11 cultivars under Georgia's specific climatic conditions. This approach was taken to establish a baseline of cultivar-specific suitability, as the development of more resilient, day-neutral genotypes will be addressed through future breeding efforts.

Accurate quantification of α- and β-acids is essential for characterizing regional differences and informing both growers and brewers. Established analytical protocols, including ASBC Hop-14 and EBC 7.7, are widely used for bitter acid determination and provide a consistent basis for comparison across studies [9,10,11]. However, careful attention to sample handling and analytical conditions remains important, as α- and β-acids are susceptible to oxidative and thermal degradation during processing [11]. Measures such as controlled storage and the use of antioxidants can help maintain analyte stability and improve data reliability, particularly when evaluating subtle compositional differences across growing environments [12,13]. In this study, a reversed-phase UHPLC approach, based on established chromatographic methods with minor optimization of experimental conditions, was applied for the determination of α- and β-acids in hops. The method was used as a reliable analytical tool to quantify α- and β-acids composition in Georgia-grown cultivars and to compare these results with values reported for the PNW.

2 Materials and methods

2.1 Georgia grown hops

Disease free hop plants were purchased from Clean Plant Center Northwest at the Washington State University Irrigated Agriculture Research and Extension Center in Prosser, Washington, in the spring of 2024. The cultivars purchased were Cascade, Cashmere, Centennial, Chinook, Cluster, Columbus, Comet, Fuggle, Magnum, Nugget, and Zeus. The hops plugs were planted at the J. Phil Campbell Sr. Research and Education Center in Watkinsville, GA (USDA Hardiness Zone 8a) in a randomized complete block design, whereas three replicates of each plant were planted randomly within three blocks (rows) [14]. Each replicate contained four individual plants (sub replicates). In the summers of 2024 and 2025, the hops plants were trained with coconut coir twine on a 5.5-meter-tall trellis system.

Throughout the growing season, plants were irrigated using a drip irrigation system to maintain adequate soil moisture. Fertilization was applied weekly via fertigation using a water soluble fertilizer (25N-5P-15K; Jack's Professional High Performance, JR Peters Inc., Allentown, PA, USA). A fertilizer concentrate was injected into the irrigation lines and flushed to achieve a total nitrogen application rate of approximately 112 kg N ha^{-1} over the growing season. No disease was observed in the Watkinsville plants, and insect pest presence was negligible.

In the summer of 2025, hop cones were harvested by hand from each plant and bulked by cultivar, representing a single time point collection within one growing season. Fresh yields were recorded as follows: Cluster (4.191 kg), Zeus (2.096 kg), Cascade (1.629 kg), Chinook (1.587 kg), Cashmere (1.192 kg), Columbus (1.049 kg), Comet (1.014 kg), Nugget (0.996 kg), Magnum (0.566 kg), Fuggle (0.324 kg), and Centennial (0.309 kg). Immediately after harvest, hop cones were dried in a low temperature forced air oven at 60 °C to a moisture content of approximately 10%, then vacuum sealed and stored at −80 °C for about seven days to limit oxidation and degradation prior to analysis.

2.2 Chemicals and reagents

The International Calibration Extract-4 (ICE-4) used for analysis of α- and β-acids (n-humulone, cohumulone, adhumulone, n-lupulone, colupulone, and adlupulone) was obtained from Labor Veritas (Zürich, Switzerland). HPLC-grade water, methanol, and acetonitrile, as well as LC-MS-grade formic acid (purity ≥ 99%), were purchased from Fisher Scientific (Waltham, MA, USA). tert-Butylhydroquinone (tBHQ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.3 Preparation of the standard solutions

Stock solutions of α- and β-acids were prepared at 100 mg·mL^{-1} by dissolving 100 mg of the extract standard mixture in 1 mL of extraction solvent (methanol/water, 80:20, v/v, containing 0.005% tBHQ and 0.1% formic acid). The stock solutions were transferred into sealed vials, purged with nitrogen, and stored at −20 °C to minimize oxidative degradation. Working standard solutions were prepared daily by appropriate dilution of the stock solution with the same extraction solvent prior to UHPLC analysis.

2.4 Sample preparation

On the day of analysis, ~40 dried hop cones of each cultivar were frozen in liquid nitrogen and immediately pulverized using a coffee grinder (Model DCG-12BC, Cuisinart, East Windsor, NJ, USA) with intermittent pulsing. The liquid nitrogen was utilized to prevent frictional heat accumulation during grinding, thereby minimizing the degradation of heat-sensitive hop acids. The powdered sample (25 mg) was transferred into 2-mL microcentrifuge tubes and extracted with 1 mL of the extraction solvent (methanol/water, 80:20, v/v, containing 0.005% tBHQ and 0.1% formic acid). The mixture was agitated using a vortex mixer for 5 min, followed by sonication in a chilled water bath (4 °C) for 15 min, and centrifugation at 14,500 × g for 5 min (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Osterode am Harz, Germany). The resulting supernatant was passed through a 0.22-µm PVDF membrane filter (Cytiva, Marlborough, MA, USA) before injection. A 10 µL aliquot was injected into the UHPLC

system. Pooled quality control (QC) samples were prepared by mixing equal volumes of all extracted samples to ensure analytical reliability within each batch. To minimize run-to-run variation, all samples were analyzed in a randomized order. Nine replicate analyses were performed to obtain accurate quantification of analytes. QC samples were injected after every fifth sample run.

2.5 UHPLC analysis

Chromatographic analysis was carried out using a Shimadzu Nexera series UHPLC system equipped with a photodiode array detector and an autosampler (Shimadzu Corp., Tokyo, Japan). Separation of α- and β-acids was achieved on an Agilent ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm; Agilent Technologies, Santa Clara, CA, USA) fitted with a guard column. The column temperature was maintained at 35 °C. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient program was as follows: 60–75% B (0–8 min), and 75–95% B (8–20 min) [15]. The system was re-equilibrated with the initial mobile phase (60% B) for 5 min before the next injection. The flow rate was 1.0 mL min^{-1}, and the injection volume was 10 µL using the autosampler. Detection was performed at 330 nm, selected to maximize overall detector response across all target analytes whose absorbance maxima fall within the 325-335 nm range, and α- and β-acids were identified by comparing their retention times with those of the ICE-4 standard. Concentrations of individual compounds were calculated using the certified composition of ICE-4, including cohumulone (10.98%), n-humulone (26.01%), adhumulone (5.5%), colupulone (13.02%), and n-lupulone + adlupulone (13.52%), with total α-acids and β-acids corresponding to 42.58% and 26.54%, respectively. Data acquisition and processing were performed using LabSolutions software (version 5.124; Shimadzu Corporation, Kyoto, Japan) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

2.6 Method validation

The analytical method was validated by assessing selectivity, linearity, precision, accuracy, sensitivity, and recovery according to that described by Singh et al. [16]. Selectivity was confirmed by comparing blank, standard, and spiked samples to ensure no interfering peaks at the analyte's retention time. Linearity was evaluated by constructing calibration curves for each compound within the concentration range of 0.01-5 mg·mL^{-1}. Precision was measured as intraday and interday repeatability by analyzing replicate samples at multiple concentrations and expressing variability as %RSD. Recovery was determined through spike-recovery experiments at different levels, with recovery calculated as measured concentration divided by the spiked amount. Sensitivity was expressed as the limit of detection (LOD) and limit of quantification (LOQ), determined from 3.3×σ/S and 10×σ/S using calibration curve slope [17,18].

3 Results

3.1 Method validation

The analytical method demonstrated satisfactory performance across all validation parameters (Table S1). The chromatograms of analytes are shown in Figure S1. All analytes exhibited excellent linearity ($R^2 \geq 0.999$; Figure S2) within the calibration range of 0.01-5 mg·mL^{-1}. Intraday and interday precision were within acceptable limits (%RSD ≤ 3.24%). Recovery values for individual analytes were 95.6% for cohumulone, 98.6% for n-humulone, 94.8% for adhumulone, 97.5% for colupulone, and 94.6% for the combined n-lupulone/adlupulone fraction. LOD values ranged from 0.87 to 1.60 µg mL^{-1}, with corresponding LOQ values of 2.63 to 4.84 µg mL^{-1}.

3.2 α- and β-acid composition of Georgia-grown hops

Significant cultivar-dependent differences in bitter acid composition (mg·g-1) were observed among the 11 Georgia-grown hop cultivars evaluated in this study (Table 1). To improve comparability with industry reporting standards, total α- and β-acid concentrations are also presented as mass-based percentages (%) on an as-is basis in Table 1. Total α-acids ranged from 37.36 ± 0.33 mg·g^{-1} in Cluster to 120.73 ± 1.31 mg·g^{-1} in Nugget, with concentrations of 118.06 ± 1.97 mg·g^{-1} in Zeus, 111.37 ± 2.02 mg·g^{-1} in Magnum, and 104.10 ± 1.14 mg·g^{-1} in Chinook, while Comet and Cascade produced α-acid concentrations of 93.74 ± 1.69 mg·g^{-1} and 73.95 ± 1.02 mg·g^{-1}, respectively. Columbus measured 86.08 ± 0.93 mg·g^{-1}, followed by Centennial (68.06 ± 0.85 mg·g^{-1}), Fuggle (55.39 ± 0.94 mg·g^{-1}), Cashmere (54.06 ± 3.13 mg·g^{-1}), and Cluster (37.36 ± 0.33 mg·g^{-1}). Total β-acids likewise varied by cultivar, ranging from 14.81 ± 0.20 mg·g^{-1} in Cluster to 42.08 ± 0.74 mg·g^{-1} in Zeus. β-acid concentrations above 39 mg·g^{-1} were also observed in Cashmere (41.00 ± 2.26 mg·g^{-1}), Cascade (40.24 ± 0.47 mg·g^{-1}), Nugget (39.29 ± 0.42 mg·g^{-1}), and Fuggle (39.58 ± 0.71 mg·g^{-1}).

3.3 Comparison with Pacific Northwest reference values

Table 1 provides reference values for total α- and β-acids (both mg·g^{-1} and mass-based percentages %) and cohumulone as a percent of α-acids (%) in PNW-grown hops [19]. These reference values were determined using the ASBC Hop-14 method, which does not separate n-humulone and adhumulone; therefore, comparisons in this study are based on summed α-acid values to ensure consistency despite differences in chromatographic resolution. Of the 11 cultivars evaluated, Cascade, Comet, and Fuggle fell within their respective PNW reference ranges (40-90, 90-120, and 30-60 mg·g^{-1}), while the remaining eight cultivars fell below their corresponding PNW benchmarks. Georgia-grown Columbus and Zeus (Table 1) measured well below PNW reference ranges of 140-180 mg·g^{-1}, while Chinook (104.10 ± 1.14 mg·g^{-1}) and Magnum (111.37 ± 2.02 mg·g^{-1}) did not reach their respective PNW ranges of 120-140 mg·g^{-1} and 120-150 mg·g^{-1}.

3.4 Environmental conditions during the growing season

To provide a standardized climatic context for the 2025 growing season, daily maximum and minimum air temperature data were obtained from the NASA POWER daily meteorological dataset for Watkinsville, GA; Yakima, WA; Salem, OR; and Nampa, ID [20]. Growing degree days were calculated over the Georgia production window, defined as April 5, 2025, when hop growth began training, through final harvest on July 14, 2025. Winter climate was characterized using heating degree days from December 1,

Table 1 Concentrations of individual and total α- and β-acids in Georgia-grown hops (n = 9) and Pacific Northwest reference values, expressed as $mg \cdot g^{-1}$ and mass-based percent (%) as-is, with cohumulone presented as a percentage of total α-acids.

	α-Acids			β-Acids		Total	Total	Total	Total	Total	Total	Total	Total		
	Cohumulone $mg \cdot g^{-1}$	n-Humulone $mg \cdot g^{-1}$	Adhumulone $mg \cdot g^{-1}$	Colupulone $mg \cdot g^{-1}$	Adlupulone +n-lupulone $mg \cdot g^{-1}$	α-acids Georgia $mg \cdot g^{-1}$	α-acids Georgia % as is	α-acids PNW $mg \cdot g^{-1}$	α-acids PNW % as is	β-acids Georgia $mg \cdot g^{-1}$	β-acids Georgia % as is	β-acids PNW $mg \cdot g^{-1}$	β-acids PNW % as is	Co-Humulone as % of α Georgia	Co-Humulone as % of α PNW
Cascade	24.34 ± 0.26	41.07 ± 0.51	8.54 ± 0.25	21.82 ± 0.30	18.42 ± 0.17	73.95 ± 1.02 f	7.40 ± 0.10 f	40-90	4.0-9.0	40.24 ± 0.47 a	4.02 ± 0.05 a	55-90	5.5-9.0	32.92 c	29-35
Cashmere	12.21 ± 0.71	34.52 ± 1.93	7.33 ± 0.49	16.38 ± 0.96	24.62 ± 1.30	54.06 ± 3.13 g	5.41 ± 0.31 g	70-100	7.0-10.0	41.00 ± 2.26 a	4.10 ± 0.23 a	30-80	3.0-8.0	22.59 i	22-24
Centennial	16.17 ± 0.04	43.39 ± 0.47	8.50 ± 0.24	8.55 ± 0.06	9.83 ± 0.12	68.06 ± 0.85 f	6.81 ± 0.09 f	70-110	7.0-11.0	18.38 ± 0.18 e	1.84 ± 0.02 e	35-55	3.5-5.5	23.77 h	23-27
Chinook	33.31 ± 0.30	59.93 ± 0.57	10.86 ± 0.27	13.41 ± 0.14	10.99 ± 0.11	104.10 ± 1.14 c	10.41 ± 0.11 c	120-140	12.0-14.0	24.40 ± 0.26 d	2.44 ± 0.03 d	30-40	3.0-4.0	32.00 d	29-35
Cluster	9.75 ± 0.06	21.51 ± 0.14	6.10 ± 0.14	6.84 ± 0.16	7.97 ± 0.04	37.36 ± 0.33 h	3.74 ± 0.03 h	60-90	6.0-9.0	14.81 ± 0.20 e	1.48 ± 0.02 e	40-60	4.0-6.0	26.10 g	36-42
Columbus	26.76 ± 0.24	47.78 ± 0.47	11.54 ± 0.21	17.85 ± 0.21	12.24 ± 0.25	86.08 ± 0.93 e	8.61 ± 0.09 e	140-180	14.0-18.0	30.09 ± 0.46 c	3.01 ± 0.05 c	45-60	4.5-6.0	31.09 e	28-35
Comet	34.31 ± 0.60	46.50 ± 0.59	12.93 ± 0.51	15.15 ± 0.24	10.85 ± 0.16	93.74 ± 1.69 d	9.37 ± 0.17 d	90-120	9.0-12.0	26.00 ± 0.40 d	2.60 ± 0.04 d	30-60	3.0-6.0	36.60 b	40-45
Fuggle	21.61 ± 0.34	25.81 ± 0.40	7.97 ± 0.20	25.14 ± 0.46	14.44 ± 0.26	55.39 ± 0.94 g	5.54 ± 0.09 g	30-60	3.0-6.0	39.58 ± 0.71 ab	3.96 ± 0.07 ab	20-40	2.0-4.0	39.02 a	25-30
Magnum	21.88 ± 0.01	76.86 ± 1.15	12.63 ± 0.53	14.12 ± 0.20	22.24 ± 0.31	111.37 ± 2.02 b	11.14 ± 0.20 b	120-150	12.0-15.0	36.36 ± 0.51 b	3.64 ± 0.05 b	60-80	6.0-8.0	19.65 j	22-28
Nugget	23.96 ± 0.24	80.09 ± 0.80	16.68 ± 0.26	16.61 ± 0.23	22.68 ± 0.19	120.73 ± 1.31 a	12.07 ± 0.13 a	130-160	13.0-16.0	39.29 ± 0.42 ab	3.93 ± 0.04 ab	40-55	4.0-5.5	19.84 j	22-30
Zeus	35.46 ± 0.63	68.31 ± 1.07	14.29 ± 0.28	24.45 ± 0.44	17.63 ± 0.30	118.06 ± 1.97 ab	11.81 ± 0.20 ab	140-180	14.0-18.0	42.08 ± 0.74 a	4.21 ± 0.07 a	45-60	4.5-6.0	30.03 f	28-35

Values are expressed as mean ± standard error (n = 9). Total α-acids represent the sum of cohumulone, n-humulone, and adhumulone, while total β-acids represent the sum of colupulone and adlupulone + n-lupulone. Percent values were calculated from $mg \cdot g^{-1}$ values on an as-is basis, where 10 $mg \cdot g^{-1}$ = 1%. Georgia-grown hop cones were dried to approximately 10% moisture prior to analysis. Different letters in each column represent statistically significant differences (Tukey's Honestly Significant Difference Test). PNW values adapted from "Hop Varieties," by Yakima Chief Hops, n.d. (https://www.yakimachief.com/hop-varieties) [19]

2024, through February 28, 2025. The indices were calculated as follows:

$$\mathrm{GDD10} = \sum^{\max} \mathrm{x}\left(\left[\frac{\mathrm{Tmax}+\mathrm{Tmin}}{2} - 10\right], 0\right)$$

$$\mathrm{HDD18.3} = \sum^{\max} \mathrm{x}\left(\left[18.3 - \frac{\mathrm{Tmax}+\mathrm{Tmin}}{2}\right], 0\right)$$

Watkinsville accumulated 1334.6 GDD10 during the production window, compared with 421.6 in Yakima, WA; 378.8 in Salem, OR; and 805.0 in Nampa, ID. Winter HDD18.3 was 1048.7 in Watkinsville, compared with 1811.4 in Yakima, 1188.2 in Salem, and 1519.8 in Nampa. Watkinsville, GA is located in USDA Hardiness Zone 8a [14]. No disease was observed on the Watkinsville plants throughout the evaluation period, and insect pest presence was negligible.

4 Discussion

4.1 Method evaluation and quantification

The optimized UHPLC method demonstrated strong analytical performance when compared with widely used standard methods such as ASBC Hop-14 and EBC 7.7, as well as more recent chromatographic approaches reported in the literature (Table S1). The complete resolution of α-acids and partial coelution of adlupulone and n-lupulone observed in this study (Figure S1) is consistent with prior reports, where structural similarity and nearly identical polarity of these β-acid isomers limit chromatographic separation even under optimized conditions [21]. Similar coelution behavior has been reported in conventional HPLC methods and is considered an inherent limitation of reversed-phase separations for these compounds [1,22]. Compared to standard isocratic methods, which typically resolve only four peaks due to coelution of n-humulone with adhumulone and n-lupulone with adlupulone, the present method achieved improved selectivity, particularly for α-acids [12]. The clear baseline separation of cohumulone, n-humulone, and adhumulone without observable matrix interference (such as prenylated flavonoids and oxidized bitter acids) highlights the advantage of the optimized gradient and sample preparation strategy [23]. The slightly lower recoveries observed for adhumulone and the lupulone fraction may reflect their greater susceptibility to degradation, while the high recovery of n-humulone confirms strong method performance for the dominant α-acid. The inclusion of an antioxidant (tBHQ) in the extraction solvent likely contributed to minimizing oxidative losses, thereby improving analyte stability and recovery [21,24]. Overall, the method performance is consistent with values reported in comparable studies [24,25].

4.2 Inter-cultivar differences

The significant differences in chemotype profiles among the Georgia-grown cultivars reveal distinct bittering and aroma characteristics. Chinook exhibited the highest ratio of α-acids to β-acids (4.27:1). Similarly, Magnum and Zeus displayed high bittering potential, with α:β ratios of 3.06:1 and 2.81:1, respectively. Conversely, Cashmere and Fuggle presented low α:β ratios (<1.4:1), consistent with the chemical profile of aroma-centric hop varieties. These α:β ratios are broadly consistent with those reported for the same cultivars grown in the PNW [4,19], indicating that the relative proportions of α- and β-acids are largely preserved under Georgia conditions despite the overall reduction in absolute acid concentrations observed in several cultivars.

In addition to total acid content, the proportion of cohumulone to total α-acids varied significantly by cultivar. While cohumulone concentration has historically been considered a critical quality parameter, with lower levels traditionally associated with a “smoother” bitterness and higher levels with a “coarser” quality [26,27], modern sensory research suggests this impact is subtle. Specifically, evidence indicates that cohumulone’s reputation for harshness may be overstated; its higher utilization efficiency can rather contribute to a smoother bitterness profile by reducing the total hop vegetable matter and associated polyphenols required during the boil [21,27,28].

Among the Georgia-grown samples, Magnum and Nugget exhibited the lowest proportions of cohumulone (19.65% and 19.84%), suggesting a high potential for clean bittering applications. Cashmere and Centennial also demonstrated low cohumulone profiles at 22.59% and 23.77%, respectively. In contrast, Fuggle (39.02%) and Cascade (32.92%) contained higher proportions of cohumulone, aligning with their established baseline profiles as traditional aroma varieties [29,30]. In these specific cultivars, this elevated cohumulone fraction is a defining trait, though its direct contribution to a ‘sharper’ bitterness is increasingly viewed as a function of the total brewing matrix and hop-to-wort ratios rather than the molecular structure of the analog alone [25,26,28].

Cashmere demonstrated a much higher standard error in total α- and β-acid accumulation than the other cultivars. This possibly reflects a lack of uniform establishment or a heightened sensitivity to regional environmental stressors during the second year of growth. This suggests that while Cashmere has the potential for commercial-grade acid production in the South, its performance consistency may require further optimization of local cultural practices.

4.3 Regional differences in α- and β-acid concentrations

Overall, while most cultivars (8 of 11) accumulated total α-acids below established PNW ranges, Cascade, Comet, and Fuggle showed comparatively stable performance in this single-season evaluation. The observation that Cascade, Comet, and Fuggle achieved commercial-grade α-acid concentrations under Georgia field conditions, while traditionally high-alpha cultivars did not, highlights significant cultivar-dependent phenotypic variability within the Southeastern production region. The stability of Comet is particularly noteworthy, as contemporary research has highlighted this cultivar’s capacity for significant chemical and sensory shifts across diverse site-specific environmental influences [5]. The results of this particular season’s harvest indicate that the Georgia Piedmont environment can support commercial-grade α-acid accumulation in select cultivars under natural photoperiods. This contrast suggests that regional benchmarks must be highly specific, as data

derived from subtropical production systems may not accurately predict cultivar performance in the distinct transitional climate of Northern Georgia.

The discrepancies in α-acid concentrations between Georgia-grown and PNW-grown hop cultivars suggest that the bittering potential for Georgia-grown hops cannot be assumed to match PNW expectations. These cultivars were specifically bred for high α-acid content, efficient iso-α-acid yields, and low hop usage in the brewhouse, making their suppressed performance under Georgia conditions particularly notable. Rather, region-specific benchmarks should be established to inform brewers of the unique traits of Georgia-grown hops. The environmental factors that may underlie these regional differences are discussed in Section 4.4.

4.4 Influence of environmental factors

A range of environmental factors influence the concentration of secondary plant compounds in Humulus lupulus. As no disease or significant pest pressure was observed during the trial (Section 3.4), the differences in bitter acid accumulation are unlikely to be attributable to biotic stress.

The standardized climate indices reported in Section 3.4 confirm that the Watkinsville site experienced substantially greater heat accumulation during the 2025 production window (1334.6 GDD10) than major PNW hop-growing regions (378.8-805.0 GDD10). High ambient temperatures have an established inhibitory effect on the accumulation of secondary metabolites, as Mozny et al. [7] demonstrated a negative, linear relationship between α-acid concentration and increasing heat in European hops. The elevated GDD accumulation at the Georgia site (approximately two to three times that of Yakima and Salem) indicates that the plants experienced sustained thermal loading during the rapid vegetative and cone developmental stages, which may have increased respiratory demand and diverted photosynthates away from the synthesis of lupulin gland constituents. Additionally, the milder winter climate (1048.7 HDD18.3 vs. 1188.2-1811.4 in PNW locations) may have implications for vernalization adequacy, though this was not directly assessed. Ultimately, the substantially elevated growing-season heat accumulation provides a possible environmental mechanism for the reduced α-acid content observed in the traditionally high-alpha cultivars.

5 Conclusions

Using a UHPLC method consistent with established standard methods (ASBC, EBC, and MEBAK), with improved α-acid resolution through gradient elution and formic acid-modified mobile phases, this study characterized bitter acid composition in 11 Georgia-grown hop cultivars. Application of this method revealed that the unique climatic conditions of the Georgia Piedmont significantly influence hop acid accumulation. While certain cultivars, such as Cascade, Comet, and Fuggle, demonstrated physiological resilience and achieved commercial-standard α-acid concentrations, high-alpha bittering varieties generally exhibited suppressed α-acid accumulation. This trend could potentially be attributed to heat-induced respiratory stress; however, further studies that analyze plant stress markers would be necessary to draw a causal relationship. These findings underscore the necessity of establishing region-specific quality benchmarks rather than relying on PNW standards. This study measured two-year-old hop plantings; hop acid concentrations may vary as the plantings mature. Preliminary yield data were recorded but should be interpreted cautiously given the establishment phase of the plantings. It is also important to note that the hop cones analyzed for this study were collected from one growing season; year-specific factors such as last frost date, temperatures, and rainfall may influence hop acid accumulation. Future research should investigate harvest timing optimization and breeding of region-specific cultivars across multiple growing seasons to further maximize the potential of this emerging crop in the Southeastern United States. Future studies should also evaluate the essential oil and aroma profiles of these cultivars to provide a complete characterization for brewers.

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Author contributions

Zachary Hutzell and Samuel O. Ogundipe contributed equally to this work.

CRediT: Zachary Hutzell: Conceptualization, Investigation, Formal analysis, Writing – Original draft, Writing – review and editing; Samuel O. Ogundipe: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing; Donglin Zhang: Conceptualization, Resources, Funding acquisition, Supervision, Project administration, Writing – Review and editing; Joon Hyuk Suh: Methodology, Resources, Supervision, Project administration, Writing – review and editing.

Declaration of interest

No potential conflict of interest was reported by the authors.

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6 References

1. Schindler, R., Sharrett, Z., Perri, M. J., & Lares, M. (2019). Quantification of α-acids in fresh hops by reverse-phase high performance liquid chromatography. ACS Omega, **4**, 3565–3570. https://doi.org/10.1021/acsomega.9b00016

2. Knoke, L., & Rettberg, N. (2026). Targeted and non-targeted analysis of hop bitter compounds using UPLC-Q-ToF. Journal of the American Society of Brewing Chemists, **1-10**. https://doi.org/10.1080/03610470.2026.2627845
3. Algazzali, V. and Shellhammer, T.H. (2016). Bitterness intensity of oxidized hop acids: Humulinones and hulupones. Journal of the American Society of Brewing Chemists, **74**, 36–43. DOI:10.1094/ASBCJ-2016-1130-01
4. Almaguer, C.; Schönberger, C.; Gastl, M.; Arendt, E.K. and Becker, T. (2014). Humulus lupulus – a story that begs to be told. A review, Journal of the Institute of Brewing, **120**, 289–314. DOI:10.1002/jib.160
5. Rosa, R.S.; Lannes, S.C.S. and Shellhammer, T.H. (2025).: Analyzing terroir influence on Comet hops: A comparison of chemical and sensory profiles of United States and Brazil, Journal of the American Society of Brewing Chemists, **83**, 298–306. DOI:10.1080/03610470.2025.2468020
6. Acosta-Rangel, A.; Agehara, S. and Rechcigl, J. (2024). Double-season production of hops (Humulus lupulus L.) with photoperiod manipulation in a subtropical climate, Scientia Horticulturae, **332**, 113177. DOI:10.1016/j.scienta.2024.113177
7. Mozny, M.; Trnka, M.; Vlach, V.; Zalud, Z.; Cejka, T.; Hajkova, L.; Potopova, V.; Semenov, M.A.; Semeradova, D. and Büntgen, U. (2023). Climate-induced decline in the quality and quantity of European hops calls for immediate adaptation measures, Nature Communications, **14**, 6028. DOI:10.1038/s41467-023-41474-5
8. Thomas, G. G., & Schwabe, W. W. (1969). Factors controlling flowering in the hop (Humulus lupulus L.). Annals of Botany, **33**(4), 781-793.
9. Biendl, M., & Anderegg, P. (2005). Modification of the method Analytica-EBC 7.7 (Alpha- and Beta-acids in Hops and Hop Products by HPLC): Submitted on behalf of the EBC Analysis Committee. BrewingScience, **58**(3/4), 32-34.
10. ASBC: American Society of Brewing Chemists. (2008). Hops **14**. α-Acids and β-Acids in hops and hop extracts by HPLC (International Method). American Society of Brewing Chemists. St. Paul, MN. https://doi.org/10.1094/ASBCMOA-Hops-14
11. Formato, A., Gallo, M., Ianniello, D., Montesano, D., & Naviglio, D. (2013). Supercritical fluid extraction of α- and β-acids from hops compared to cyclically pressurized solid-liquid extraction. Journal of Supercritical Fluids, **84**, 113–120. https://doi.org/ 10.1016/j.supflu.2013.09.021
12. Baker, G.A.; Danenhower, T.M.; Force, L.J.; Petersen, K.J. and Betts, T.A. (2008): HPLC analysis of α- and β-acids in hops, Journal of Chemical Education, **85**, 954.
13. Duarte, L. M., Aredes, R. S., Amorim, T. L., de Carvalho Marques, F. F., & de Oliveira, M. A. L. (2022). Determination of α-and β-acids in hops by liquid chromatography or electromigration techniques: A critical review. Food chemistry, **397**, 133671. https://doi.org/10.1016/j.foodchem.2022.133671
14. U.S. Department of Agriculture, Agricultural Research Service 2023: USDA plant hardiness zone map, https://planthardiness.ars.usda.gov/, accessed 17 November 2025.
15. Teng, C. E., Wang, Y. M., Li, T. H., & Chen, S. F. (2024). Quantification of hop-derived bitter compounds in beer using liquid chromatography mass spectrometry. Journal of the American Society for Mass Spectrometry, **35**(4), 746-755.
16. Singh, S., Sharma, H., Kumar, V., Gupta, G., Patel, S., Patel, A., ... & Singh, S. K. (2025). Method development and validation on RP-HPLC method for estimation of xanthohumol in nanostructured lipid carriers drug delivery systems. Journal of Chromatography B, **1252**, 124437. https://doi.org/10.1016/j.jchromb.2024.124437
17. Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles of Young Scientists, **2**(1), 21–25. https://doi.org/10.4103/2229-5186.79345
18. Tofană, M., Mudura, E., Muste, S., Socaci, S. A., & Mureşan, C. (2013). The validation of the HPLC Hop Bitter acids method. J. Agroaliment. Processes Technol., **15**(1), 50–54.
19. Yakima Chief Hops n.d.: Hop varieties, https://www.yakimachief.com/hop-varieties, accessed 11 December 2025.
20. NASA Prediction of Worldwide Energy Resources (POWER) Project 2025: Daily meteorological data for Watkinsville, GA; Yakima, WA; Salem, OR; and Nampa, ID, NASA Langley Research Center POWER Project, https://power.larc.nasa.gov/, accessed 8 May 2026
21. Arruda, T. R., Pinheiro, P. F., Silva, P. I., & Bernardes, P. C. (2021). A new perspective of a well-recognized raw material: Phenolic content, antioxidant and antimicrobial activities and α- and β-acids profile of Brazilian hop (Humulus lupulus L.) extracts. LWT - Food Science and Technology, **141**, Article 110905. https://doi.org/10.1016/j.lwt.2021.110905
22. Culik, J., Jurkova, M., Horak, T., Cejka, P., Kellner, V., Dvorak, J., ... Roth, M. (2009). Extraction of bitter acids from hops and hop products using pressurized solvent extraction (PSE). Journal of the Institute of Brewing, **115**(3), 220–225. https://doi.org/10.1002/J.2050-0416.2009.TB00372.X
23. Roj, E., Tadic, V. M., Misic, D., Zizovic, I., Arsic, I., Dobrzynska-Inger, A., & Kostrzewa, D. (2015). Supercritical carbon dioxide hops extracts with antimicrobial properties. Open Chemistry, **13**(1), 1157–1171. https://doi.org/10.1515/chem-2015-0131
24. Prencipe, F. P., Brighenti, V., Rodolfi, M., Mongelli, A., Dall'Asta, C., Ganino, T., Bruni, R. & Pellati, F. (2014). Development of a new high-performance liquid chromatography method with diode array and electrospray ionization-mass spectrometry detection for the metabolite fingerprinting of bioactive compounds in Humulus lupulus L. Journal of Chromatography A, **1349**, 50–59. https://doi.org/ 10.1016/j.chroma.2014.04.097.
25. Sartori, L. O., Souza, A. A., Bragagnolo, F. S., Fortuna, G. C., Bonfim, F. P. G., Sarnighausen, V. C. R., ... Funari, C. S. (2022). An efficient, fast, and green procedure to quantify α- and β-acids and xanthohumol in hops and their derived products. Food Chemistry, **373**, 131323. https://doi.org/10.1016/j. foodchem.2021.131323
26. Schönberger, C. and Kostelecky, T. (2011). 125th anniversary review: The role of hops in brewing, Journal of the Institute of Brewing, **117** (2011), 259–267. DOI:10.1002/j.2050-0416.2011.tb00471.x
27. Protsenko, L.; Ryzhuk, S.; Liashenko, M.; Shevchenko, O.; Litvynchuk, S.; Yanse, L. and Milosta, H. (2020). Influence of alpha acids hop homologues of bitter and aromatic varieties on beer quality, Ukrainian Food Journal, **9**, 425–436. DOI:10.24263/2304-974x-2020-9-2-13
28. Shellhammer, T. H. (2009). Hop components and their impact on the bitterness quality of beer. In T. H. Shellhammer (Ed.), Hop Flavor and Aroma: Proceedings of the 1st International Brewers Symposium 167–179. St. Paul, MN: American Society of Brewing Chemists.
29. Afonso, S.; Arrobas, M. and Rodrigues, M.A. (2021). Agronomic and chemical evaluation of hop cultivars grown under Mediterranean conditions, Spanish Journal of Agricultural Research, **19**, e0904. DOI:10.5424/sjar/2021193-17528
30. Padgitt-Cobb, G.L.; Oestreich, R.L.; Henning, S.R.; Kingan, J.M.; Peluso, P.; Rank, D. and Hendrix, J.C. (2023). An improved assembly of the

"Cascade" hop (Humulus lupulus) genome uncovers signatures of molecular evolution and refines time of divergence estimates for the Cannabaceae family, The Plant Genome, **16**, e20275. DOI:10.1002/tpg2.20275

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Supplementary Data

Table S1 Method evaluation parameters including linearity, precision, accuracy, and detection limits for α- and β-acids by UHPLC analysis.

Compound Name	R^2	Interday %RSD	Intraday %RSD	% Recovery	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Cohumulone	1	1.02	1.11	95.6	1.26	3.82
n-Humulone	1	3.24	2.25	98.6	0.87	2.63
Adhumulone	0.999	2.90	3.23	94.8	1.31	3.97
Colupulone	1	2.31	2.14	97.5	1.11	3.37
Adlupulone+n-lupulone	1	1.60	1.26	94.6	1.60	4.84

R^2 represents the coefficient of determination of the calibration curve. Interday and intraday %RSD indicate method precision, while % recovery reflects accuracy. LOD and LOQ correspond to the limits of detection and quantification, respectively, expressed in µg mL^{-1}

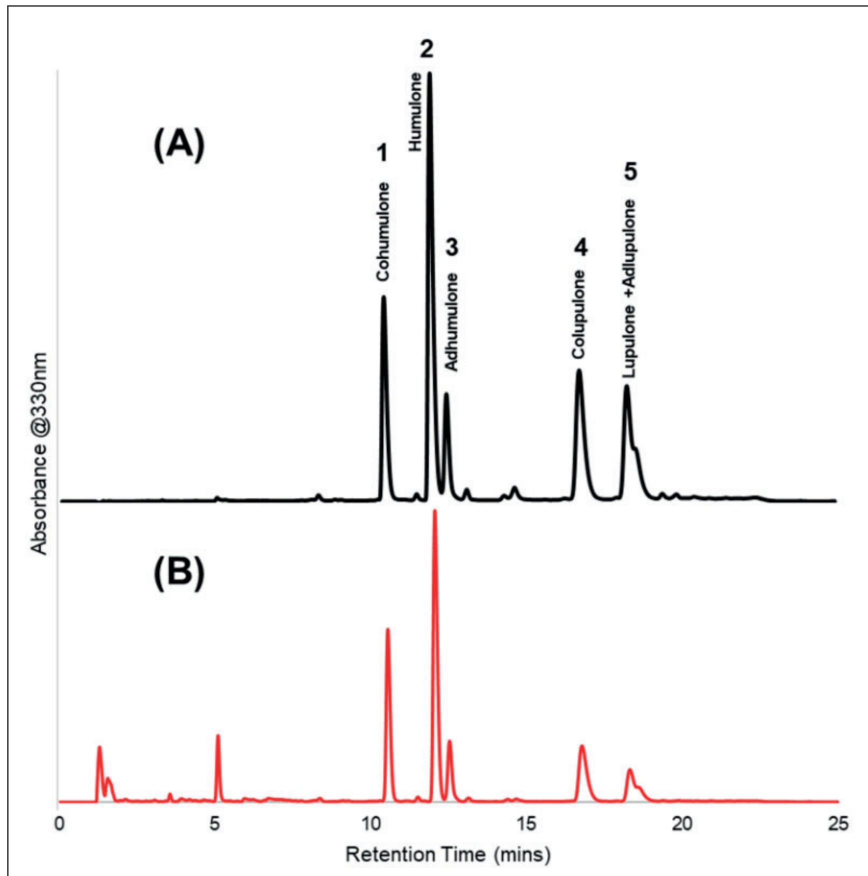

Fig. S1 **Representative UHPLC chromatograms of α- and β-acids in the ICE-4 standard mix (A) and a hop sample (B). Peaks: (1) cohumulone, (2) n-humulone, (3) ad-humulone, (4) colupulone, (5) adlupulone + n-lupulone.**

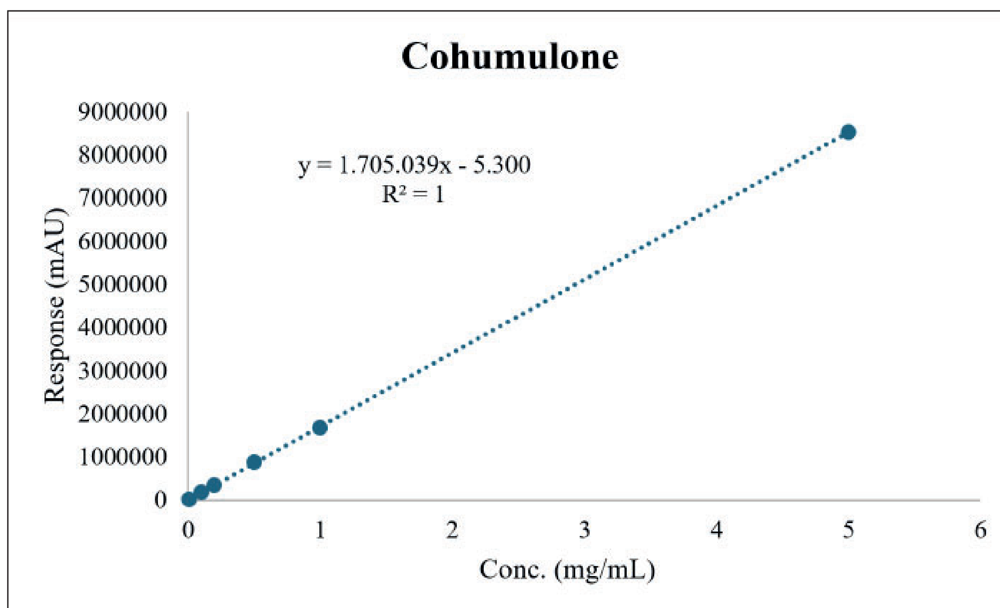

Fig. S2A

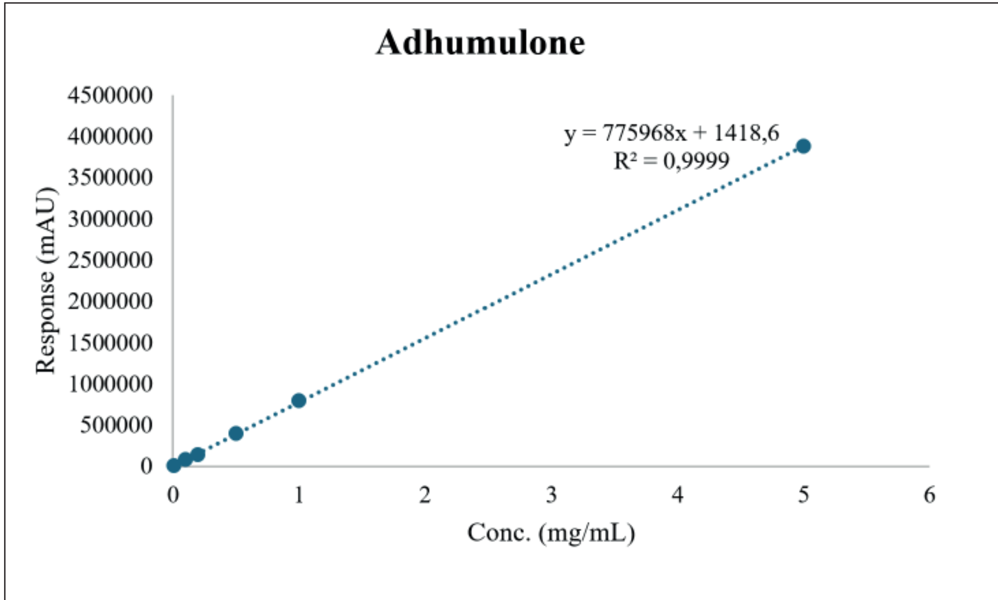

Fig. S2B

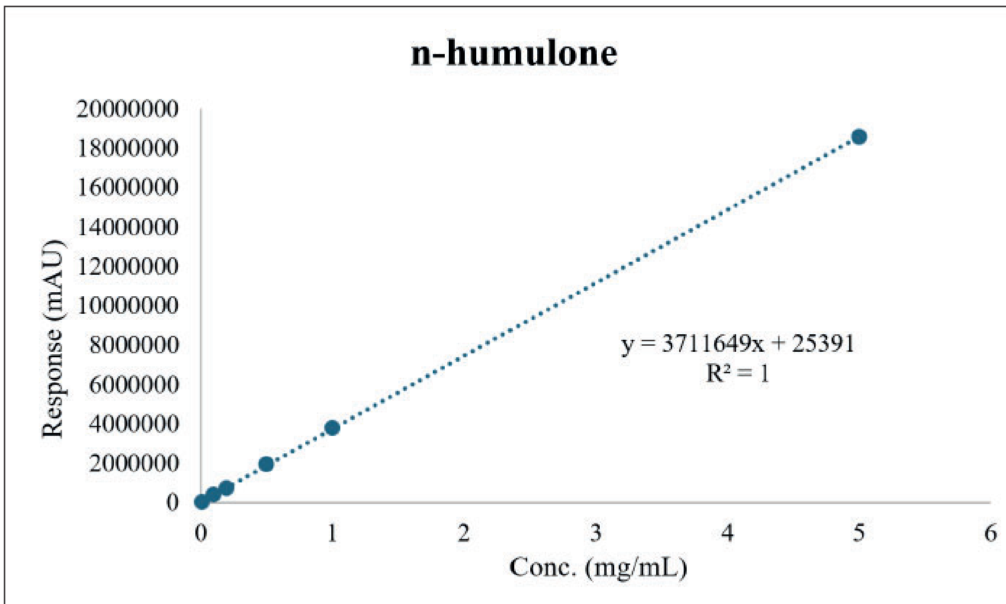

Fig. S2C

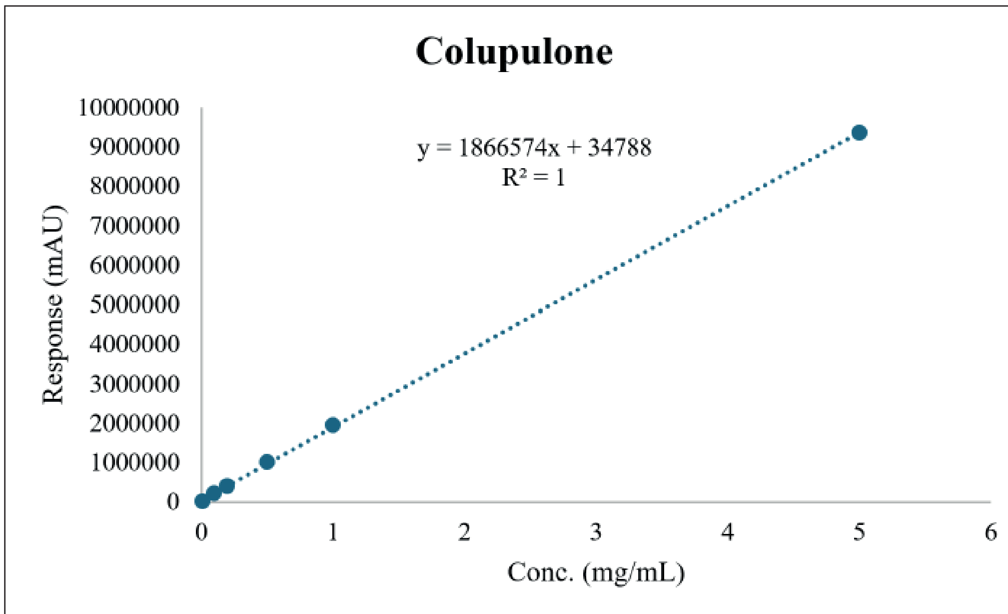

Fig. S2D

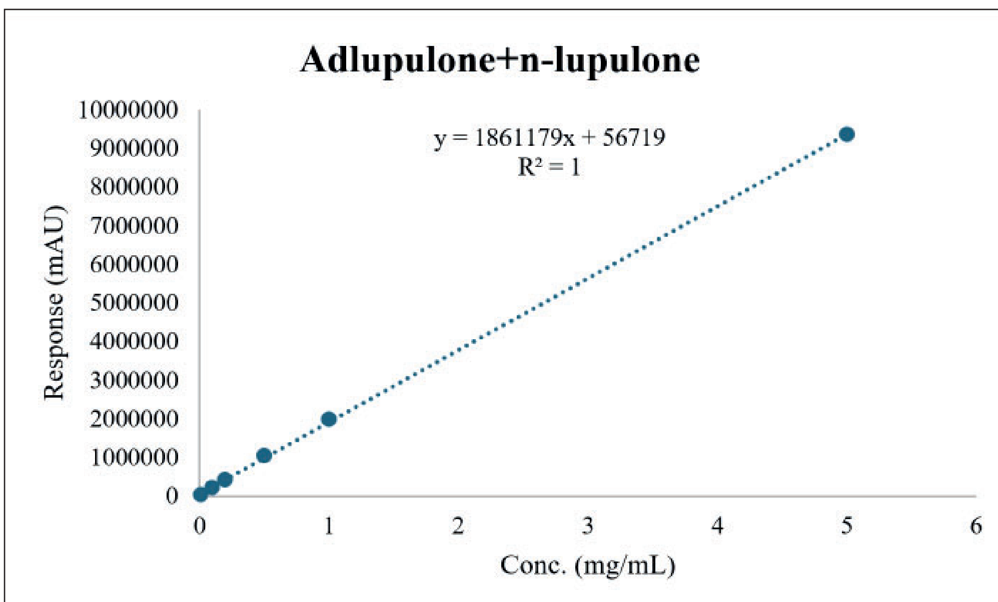

Fig. S2E

Fig. S2 **Calibration curves for (A) cohumulone, (B) adhumulone, (C) n-humulone, (D) colupulone, and (E) adlupulone + n-lupulone**