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Assessment of the phytochemical profiles of novel hop (*Humulus lupulus* L.) cultivars: A potential route to beer crafting

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ABSTRACT

This study investigated the volatile phytochemical diversity of 30 samples obtained from experimental hybrid and commercial *H. lupulus* L. plants. Essential oils distilled from these samples were analysed by high resolution gas chromatography coupled with accurate mass time-of-flight mass spectrometry (GC–accTOFMS). A total of 58 secondary metabolites, mainly comprising 18 esters, 6 monoterpene hydrocarbons, 2 oxygenated monoterpenes, 20 sesquiterpene hydrocarbons, 7 oxygenated sesquiterpenes, and 4 ketones, were positively or tentatively identified. A total of 24 metabolites were detected in all samples, but commercial cultivars (selected for brewing performance) had fewer compounds identified compared to experimental genotypes. Chemometrics analyses enabled distinct differentiation of experimental hybrids from commercial cultivars, discussed in terms of the different classes of compounds present in different genotypes. Differences among the mono- and sesquiterpenoids, appear to be related to either: i) the genetic origin of the plants; or ii) the processes of bioaccumulation of the identified secondary metabolites.

1. Introduction

Hop (*Humulus lupulus* L.), a deciduous, perennial, dioecious climbing plant of the Cannabaceae family, is an iconic crop cultivated on a relatively restricted land base worldwide (Meier, 2017). Hop cones (the fruit) mature and are harvested in early autumn, and the bioaccumulation of secondary metabolites during fruit maturation is closely linked to the processes of senescence. The primary use of hop products is in the brewing industry, where they are an essential ingredient to impart flavour (bitterness and aroma) to beer (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014). Although a large number of hop varieties are commercially available (Briggs, Boulton, Brookes, & Stevens, 2004), the changing structure of the brewing industry (approximately 6300 craft breweries were recognised in the United States in 2017) (Brewers Association, 2018) and increasing consumption of hop (approximate 39,500 tonnes of hop were harvested by United States hop producers in 2016) (French, 2017) have given rise to an unprecedented demand for the development of differentiated flavour options *via* new *H. lupulus* L. cultivars.

Hop cultivars can confer distinct flavour and aroma characteristics to beer, which are attributable to the accumulated phytochemistry of hop cones (Almaguer et al., 2014). A complex pool of volatile secondary metabolites comprised of hydrocarbons (monoterpenes, sesquiterpenes, and aliphatics), oxygenated compounds (alcohols, epoxides, esters, and ketones), and sulfur-containing compounds, accumulate in hop cones, which contributes to the unique organoleptic properties of particular cultivars noted in beer (Sharpe & Laws, 1981; Steenackers, De Cooman, & De Vos, 2015). Characteristic essential oil profiles extracted from hop cones have been used to identify cultivars, determine genetic variability or diversity, and interpret aroma properties (Kovačevič & Kač, 2002;

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Patzak, Nesvadba, Henychová, & Krofta, 2010). Hence, the chemical patterns of essential oils derived from commercial and wild hop varieties have been the object of many studies (Gonçalves et al., 2014; Mongelli et al., 2015; Van Opstaele, De Causmaecker, Aerts, & De Cooman, 2012). Relatively few previous studies link phytochemical information directly to genetic relationships, horticultural or agricultural processes, or the biology of accumulation of odour-active secondary metabolites. Studies have commonly focused on a relatively limited number of genotypes and established cultivars, meaning that the observed phytochemical variation has been determined by the brewing application (e.g., bittering, flavour or aroma) that studied hop cultivars were developed to serve (Dresel, Vogt, Dunkel, & Hofmann, 2016; McAdam, Vaillancourt, Koutoulis, & Whittock, 2014). This bias represents a limitation in ability to understand the process of bioaccumulation of desirable secondary metabolites, or the difference between desirable and undesirable chemotypes in hop for particular brewing applications.

The range and/or abundance of secondary metabolites in relevant industrial crops are influenced by both environment (in the broadest sense – incorporating horticultural and processing conditions) and genotype (Hall et al., 2002; Roessner et al., 2001). However, in the case of hop, it is clear that genotype is the major determinant of the chemical composition of a particular cultivar, provided that planting material, growing, harvesting and post-harvest processes are consistent and well-controlled (Eyres & Dufour, 2009; Kovačevič & Kač, 2002). Application of modern bioinformatic techniques can improve understanding of biosynthesis and accumulation of flavour-relevant secondary metabolites, and their utilisation or transformation during various brewing processes. It is important to differentiate between compounds that can be used to characterise hop genotypes, compounds that are odour-active in hop, compounds that are precursors to aroma-active compounds in beer, and compounds that are odour-active in beer. A detailed understanding of the relevant biological processes will provide the opportunity to target accumulation and retention of relevant flavour compounds through new cultivar development, horticultural, and brewing processes.

This study describes a detailed chemical characterisation of essential oil distilled from experimental hybrid hop genotypes and commercial Australian hop cultivars grown in the Southern Hemisphere (Tasmania, Australia), using high resolution gas chromatography with accurate mass time-of-flight mass spectrometry (GC–accTOFMS). The variation in phytochemical composition among the analysed samples was critically evaluated and interpreted in terms of their genetic and biogeographical origins, selection, and in light of understanding of the biosynthetic pathways that result in accumulation of secondary metabolites in hop essential oil. This study provides valuable information for the continued development of distinctive hop character in beer.

2. Materials and methods

2.1. Chemicals and reagents

Analytical reagent grade dichloromethane was purchased from Merck (Darmstadt, Germany). A series of *n*-alkanes (C_7–C_{30}) was purchased from Sigma-Aldrich (St. Louis, MO). α-Pinene (97%), β-pinene (97%), β-myrcene (≥95%), limonene (96%), γ-terpinene (≥95%), β-linalool (97%), caryophyllene (≥98%), and humulene (≥96%) were provided by Australian Botanical Products (Hallam, Australia).

2.2. Hop samples

In January 2008, as part of the study conducted by McAdam et al. (2014) an open pollinated seedlot was collected from the European landrace variety Hersbrucker (held in the *H. lupulus* germplasm collection at Bushy Park Estates, Tasmania) (Howard et al., 2011). This seedlot produced a male plant (genotype code 08-177-002), pollen from which was subsequently (January 2011) used to pollinate the cultivar Hersbrucker. This cross produced a seedlot (cross code 11-005) comprising 166 progeny, that was clonally replicated (*via* vegetative propagation) and deployed (along with replicates of both the female and male parents of the cross) into trials of two replicates of single plant plots in each of the major hop growing localities in south eastern Australia (Bushy Park Estates, Tasmania: latitude −42.706292, longitude 146.900924; and Rostrevor Hop Gardens, Victoria: latitude −36.624522, longitude 146.822234) in December 2011.

Whole, machine-harvested hop cones were collected from 17 experimental hop genotypes (18 samples, from 18 individual plants; with two separate plants of the same genotype 11-005-060 sampled) were collected from the Tasmanian trial site. Whole, machine-harvested hop cones from commercial cultivars HPA-035, Cascade, Ella, Enigma, Galaxy, Helga, Pride of Ringwood, Summer, Super Pride, and Willamette were collected from commercial operations at Bushy Park, Tasmania. Whole, machine-harvested hop cones from commercial cultivars Topaz and Vic Secret were collected from commercial operations at Rostrevor Hop Gardens, Victoria. All samples were harvested in March 2015, and were dried under standard conditions (60 °C for *ca.* 10 h). Following drying, all samples were sealed in carbon dioxide back-flushed oxygen barrier foil packages and preserved at ~2 °C until required for analysis. A more detailed description of the investigated experimental and commercial hop cultivars is shown in Table 1.

2.3. Hydrodistillation

Collected hop cones (approx. 50 g) were dried and pulverised, and submitted to hydrodistillation (1.25 L deionised water) using a Clavenger apparatus for 3 h. Essential oils were collected from the condenser and stored at −20 °C when not in use. A 1:100 dilution of the distillate in dichloromethane was prepared prior to GC analysis. Each sample was analysed in triplicate.

2.4. GC–accTOFMS analysis

Separations were conducted using an Agilent 7890A GC system coupled with a 7200 series quadrupole-time-of-flight mass spectrometer (QTOFMS; Agilent Technologies, Mulgrave, Australia). Chromatographic separation was performed using a BPX5 column (Trajan Scientific and Medical, Ringwood, Australia) of dimension 60 m × 0.25 mm I.D. × 0.25 µm film thickness. The end of the column was connected to the QTOFMS *via* a deactivated fused-silica column (DFS; 0.3 m × 0.1 mm I.D.). The MS transfer line temperature was set at 280 °C. Helium was used as the carrier gas (99.999% purity) at a constant flow rate of 1.3 mL/min. The chromatographic conditions were: oven temperature program, 40 °C (hold 1 min) heated at 3 °C/min to 270 °C (hold 3 min); injector temperature was 200 °C; injection volume was 1 µL of the prepared diluted sample (as described in Section 2.3) with a split ratio of 10:1. The QTOFMS was operated in a total transfer of ion (TTI) mode through the quadrupole sector. Ion source temperature, emission current, and electron ionisation voltage were set at 280 °C, 25 µA, and 70 eV, respectively. A mass range of 40–500 Da with TOF mass resolution of 2 GHz extended dynamic range was applied in all experiments. This system was used for its accurate mass capability. Since TTI mode was employed, the quadrupole sector was not used to provide further ion filtering, and so for the purposes of this study, the Q sector will not be considered. This is referred to hereafter as accTOFMS.

2.5. Chromatographic data handling

MassHunter Version B.06.00 (Agilent Technologies) was used for data acquisition and processing. Acquired MassHunter data were exported in .csv format for data display using OriginPro 8 SR2 software Version 8.0891 (Origin, Northampton, MA). Identification of the individual components was based on: (a) comparison of the obtained