Food Chemistry 205 (2016) 212–220

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The impact of hop bitter acid and polyphenol profiles on the perceived bitterness of beer

Olayide Oladokun [a], Amparo Tarrega [a], Sue James [b], Katherine Smart [b], Joanne Hort [a], David Cook [a,*]

[a] International Centre for Brewing Science, Bioenergy and Brewing Science Building, University of Nottingham, School of Biosciences, Division of Food Science, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[b] SABMiller Plc, SABMiller House, Church Street West, Woking, Surrey GU21 6HS, UK

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 7 March 2016
Accepted 8 March 2016
Available online 8 March 2016

Chemical compounds studied in this article:
Protocatechuic acid (PubChem CID:72)
Catechin (PubChem CID:73160)
Epicatechin (PubChem CID:72276)
Caffeic acid (PubChem CID:689043)
Vanillic acid (PubChem CID:8468)
Ferulic acid (PubChem CID:445858)
p-Coumaric acid (PubChem CID:637542)
Cinnamic acid (PubChem CID:444539)
4-Hydroxyphenylacetic acid (PubChem CID:127)
Sinapic acid (PubChem CID:637775)

Keywords:
Beer
Phenolic acids
Total polyphenol content
Hop acids
Humulinones
Tetrahydro-iso-humulones
Bitterness quality

ABSTRACT

Thirty-four commercial lager beers were analysed for their hop bitter acid, phenolic acid and polyphenol contents. Based on analytical data, it was evident that the beers had been produced using a range of different raw materials and hopping practices. Principal Components Analysis was used to select a sub-set of 10 beers that contained diverse concentrations of the analysed bitter compounds. These beers were appraised sensorially to determine the impacts of varying hop acid and polyphenolic profiles on perceived bitterness character. Beers high in polyphenol and hop acid contents were perceived as having 'harsh' and 'progressive' bitterness, whilst beers that had evidently been conventionally hopped were 'sharp' and 'instant' in their bitterness. Beers containing light-stable hop products (tetrahydro-iso-α-acids) were perceived as 'diminishing', 'rounded' and 'acidic' in bitterness. The hopping strategy adopted by brewers impacts on the nature, temporal profile and intensity of bitterness perception in beer.

1. Introduction

Bitterness is an important flavour character of foods and beverages such as coffee, nuts, fruits and beer (Lesschaeve & Noble, 2005). Whereas the bitterness flavour of tea and red wine have been attributed mainly to flavonoid phenols, approximately 80% of beer bitterness is derived from the addition of hops (*Humulus lupulus*) during the 'boiling stage' of the brewing process (Arrieta, Rodríguez-Méndez, De Saja, Blanco, & Nimubona, 2010; Caballero, Blanco, & Porras, 2012). The lupulin glands of female hop cones contain soft resins rich in phloroglucinol derivatives, namely α-acids (cohumulone, humulone, adhumulone) and β-acids (colupulone, lupulone, adlupulone). These acids undergo thermal isomerisation to give iso-α-acids, the major bitter compounds in beer (Haseleu et al., 2010). Upon isomerisation, each iso-α-acid congener is present as trans/cis stereoisomers with a ratio of approximately 3:7 in conventionally hopped beers (Schönberger & Kostelecky, 2011). In recent years beer-bittering practice has diversified, with the development and usage of hop products in a variety of different forms, and with varied points of addition to the brewing process (e.g. kettle addition, post-fermentation bittering products, or dry hopping, which is feasible at a number of different points). One such product is

* Corresponding author.
E-mail address: david.cook@nottingham.ac.uk (D. Cook).

http://dx.doi.org/10.1016/j.foodchem.2016.03.023
0308-8146/

pre-isomerised iso-α-acids, widely available as an aqueous extract or in pellet form, which are prepared from the chemical isomerisation of α-acids outside of the brewhouse. These hop products usually have higher levels of cis-isomers relative to trans-isomers thus, giving a lower trans/cis ratio (Schmidt et al., 2014). Bitterness can also be achieved by the use of chemically reduced derivatives of iso-α-acids, so called light stable hop products such as tetrahydro-iso-humulones (tetra) and hexahydro-iso-humulones (hexa) which are prepared by hydrogenation and reduction reactions, respectively. Advanced hop products are popular among brewers because they offer added flexibility in terms of their usage, and can be added downstream of the brewing process (De Keukeleire, 2000).

Furthermore, hops available in various forms (cones, pellets, plugs) can be added at different stages of the brewing process. Some brewers also soak hops in beer during fermentation or conditioning to improve beer aroma in a technique known as 'dry-hopping'. Dry-hopping imparts oxidised α-acids (known as humulinones) to beer. Humulinones levels of 0.2–0.5% w/w have been reported in hop leaves and pellets (Cocuzza & Mitter, 2008; Negri, di Santi, & Tabach, 2010; Wolfe, 2012). In addition to α-acids, hops are also a source of polyphenols in beer although the amount of polyphenols present in beer will depend on hop variety, form and the point at which the hops are added during the brewing process. Furthermore, depending on hopping levels, brewing malt usually represents the major source of polyphenols in beer (Aron & Shellhammer, 2010; Callemien & Collin, 2009).

Polyphenols contribute to bitterness, colour, body, and astringency in beer and other beverages such as tea and wine, (Collin, Jerkovic, Bröhan, & Callemien, 2013) and have been recognised to influence the acceptance of beverages (Drewnowski & Gomez-Carneros, 2000). In beer they act as antioxidants, preventing oxidative degradation of beer whilst also providing potential health benefits to consumers through their inhibitory activity on certain mutagens and carcinogens (Floridi, Montanari, Marconi, & Fantozzi, 2003). These compounds are diverse in chemical structure and can be divided into groups consisting of simple hydroxycinnamic and hydroxybenzoic acid derivatives (phenolic acids), flavanols, flavanol glycosides and prenylated flavonoids (Goiris et al., 2014). Flavanols are of particular interest to brewers because they form protein–polyphenol complexes, leading to the formation of haze or turbidity in beer – brewers consequently remove them by cold filtration or polypvinylpyrrolidine (PVPP) treatment (Garcia, Grande, & Gándara, 2004). However, PVPP treatment is not selective for the removal of haze active polyphenols only – leading to losses of other polyphenols that are potentially beneficial to the flavour and stability of beer (Aron & Shellhammer, 2010; Mikyška, Hrabak, Hašková, & Šrogl, 2002).

The oral sensation of astringency is perceived as a drying, puckering or rough mouth-feel, resulting from the precipitation of proline-rich proteins in saliva by polyphenols (McLaughlin, Lederer, & Shellhammer, 2008). Several phenolics including ferulic acid, *p*-coumaric acid and protocatechuic acid have also been noted to elicit astringency (Callemien & Collin, 2009). Flavanol monomers such as catechin and epicatechin were found to be more bitter than astringent (Drewnowski & Gomez-Carneros, 2000; Peleg, Gacon, Schlich, & Noble, 1999).

It is widely accepted within the brewing industry that the bitterness characteristics of beers differ due to factors not determined using the simplistic analytical measurement of bitterness units (BU). It is anticipated that this might relate to the diversity of hop products and hopping strategies employed across the industry and the impacts which this has on the relative concentrations of the array of compounds contributing to bitterness perception. Whilst there is some knowledge of the individual bitterness qualities which hop acid isomers impart to beer (Fritsch & Shellhammer, 2009), the links between hopping practice, bittering congener profile and the perceived bitterness characteristics of beers remains poorly understood. In this study we analysed the major hop acid isomers and polyphenolic compounds present in 34 commercially significant lager beers sourced from around the world. Having thus established the analytical bittering profiles of these beers, 10 beers, which varied significantly in the congeners present, were selected for sensory evaluation. A sensory lexicon for beer bitterness was developed to adequately reflect the diversity of bitterness experienced by the panel and was used to rate beer bitterness characteristics. Finally, correspondence analysis of the sensory data set was used to explore links between the bitterness congener profiles and perceived bitterness character of beers. This study thus represents a significant step towards understanding how to control this important flavour attribute of beers.

2. Materials and methods

2.1. Materials

34 fresh commercial lager beers were sourced from 17 countries over 4 continents and analysed within 8 weeks of production. For reasons of confidentiality the beers are not identified but the countries from which they were sourced are as follows: Australia (2), Belgium (1), Cuba (1), Czech Republic (6), Denmark (1), France (1), Germany (2), Hungary (1), Italy (2), Netherlands (3), Poland (2), Peru (1), Romania (1), South Africa (3), Turkey (1), UK (2) and USA (4).

2.2. Chemicals and reagents

Hydroquinone (99%), catechin (99%), epicatechin (98%), 4-hydroxybenzoic acid (99%), caffeic acid (95%), vanillic acid (97%), syringic acid (95%), *p*-coumaric acid (98%), sinapic acid (98%), ferulic acid (99%), 2,5-dihydroxybenzoic acid (98%), gallic acid (98%), cinnamic acid (98%), salicylic acid (99%), 1,2-didydroxybenzene (99%), homovanillic (99%), gentisic acid (98%) and chlorogenic acid (99%) were all purchased from Sigma–Aldrich (UK). Protocatechuic acid (99.6%) was acquired from HWI analytic (Germany). Ethyl benzoate, isooctane and methanol (all HPLC grade) as well as orthophosphoric acid 85% (ASC grade) were purchased from VWR (UK). Reverse osmosis (RO) water was obtained from a Milli-Q water purification system by Millipore. Carboxymethylcellulose (CMC), ethylenediamine tetraacetic acid (EDTA), ammonia and ferric reagent solutions were all technical grade chemicals from VWR (UK). For humulinone synthesis, CO_2 extract of α-acid resin (86%) was kindly donated by Botanix, Paddock Wood, Kent. Cumene hydroperoxide (80% technical grade), diethyl ether, sodium bicarbonate, hexane, phosphoric acid and hydrochloric acid (HCl) were all from Sigma–Aldrich (UK) and of ASC reagent grades.

Iso-α-acid standard (ICE-3) containing trans-isocohumulone, trans-isohumulone, trans-isoadhumulone (62.3% w/w), α- & β-acid (44.64%, 24.28% w/w), and tetra standard (99.3% w/w) were purchased from Labor Veritas Co. (Switzerland).

2.3. Instrumentation

HPLC analysis was carried out on a Waters Alliance 2695 instrument equipped with a column heater and a membrane degasser. Detection was achieved with a UV detector and peak areas were processed with the operating HPLC software (Empower 2). Separation of polyphenols and hop acids was achieved with a Purospher STAR rp-18 endcapped column (250 × 4.6 mm, 3 μm) from Merck