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Beer produced via hydrodynamic cavitation retains higher amounts of xanthohumol and other hops prenylflavonoids

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ABSTRACT

Some of the most valuable bioactive compounds in beer comes from hops polyphenols, mainly flavonoids, some of which are unique to inflorescences of that flowering plant. Although far from pharmacologically relevant concentrations, low doses of xanthohumol and related prenylflavonoids found in beer contribute to the overall antioxidant activity of the product, as well as to significant chemopreventive action about certain diseases, such as cardiovascular, neurodegenerative, and some cancer types. Hence, the efforts to explore both ingredients and brewing methods aimed at enhancing the concentration of such bioactive compounds. In this study, a novel brewing method assisted by hydrodynamic cavitation was experimented, proving its ability to retain or generate higher amounts of xanthohumol, desmethylxanthohumol and 6-geranylnaringenin. Operational parameters, concerning hops processing, and leading to the enhanced retention, or generation, of the considered prenylflavonoids, were found to be common to all those same compounds. As well, basic mechanisms were hypothesized, such as increased extraction from hops, reduced adsorption to insoluble malt proteins, and reduced isomerization. The results expand recent evidence about enhanced extraction of bioactive compounds by processes based on hydrodynamic cavitation, as well as add to already proven benefits of hydrodynamic cavitation to the brewing processes.

1. Introduction

First detected in beer in 1999 (Stevens, Taylor, & Deinzer, 1999), xanthohumol (3'-[3,3-dimethyl allyl]-2′,4′,4-trihydroxy-6′-methoxychalcone, hereinafter also denoted as XN, molecular formula $C_{21}H_{22}O_5$) is a prenylated flavonoid secreted by hop (*Humulus lupulus*) inflorescences. XN is unique to hops, the most abundant polyphenol found in hard resins in the hop lupulin glands (Almaguer, Schönberger, Gastl, Arendt, & Becker, 2014), as well as shows excellent biological and molecular activity (Venturelli et al., 2016).

Based on the increasingly understood pharmacological profile (Liu et al., 2015), preclinical evidence proved XN's action as antithrombotic (Xin et al., 2017), hepatoprotective (Weiskirchen, Mahli, Weiskirchen, & Hellerbrand, 2015), anti-atherosclerotic (Hirata et al., 2017), and anti-carcinogenic, both *in vitro* (Ferk et al., 2010; Gerhäuser, 2005; Karabin, Hudcova, Jelinek, & Dostalek, 2015), and based on early clinical evidence (Pichler et al., 2017).

Although beer contains far less than the pharmacological doses of XN (16.9 mg/kg to 1 g/kg), the respective dietary intake of XN and related prenylflavonoids has been associated with distinct chemopreventive effects on certain cancer types (Blanquer-Rosselló, Oliver, Valle, & Roca, 2013), contributing to the healthy effects of moderate beer consumption (de Gaetano et al., 2016). Indeed, more in general, regular and long-term dietary intake of antioxidant-rich foods was recently proven to provide distinctly positive health effects (Soccio et al., 2018).

The above evidence is quite relevant, because beer is the worldwide most consumed alcoholic beverage, with about 200 billion liters per year (Stack, Gartland, & Keane, 2016), motivating the efforts to

Abbreviations: 3′;-GCN, 3′;-geranylchalconaringenin; 6-GN, 6-geranylnaringenin; BIAB, Brew In A Bag; CN, Cavitation Number; DMX, desmethylxanthohumol; EBC, European Brewery Convention; HESI, heated electrospray; HC, Hydrodynamic Cavitation; IBU, International Bitterness Unit; MS, mass spectrometry; SRM, Standard Reference Method; UHPLC, ultra-high performance liquid chromatography; XN, xanthohumol

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produce XN-enriched beers. XN concentrations as high as 3.5 mg/L (Magalhães, Dostalek, Cruz, Guido, & Barros, 2008), or even 10 mg/L, were achieved, without negatively affecting fermentation (Magalhães et al., 2011). XN-enriched beers showed improved shelf-life (Karabín, Hudcová, Jelínek, & Dostálek, 2016), as well as, sometimes, aroma and taste (Dresel, Vogt, Dunkel, & Hofmann, 2016). Moreover, unclarified craft beers, retaining most of naturally extracted polyphenols, showed higher overall antioxidant activity (Marques et al., 2017).

XN and other prenylated hops' flavonoids, such as desmethylxanthohumol (DMX, molecular formula $C_{20}H_{20}O_5$), the latter also unique to hops (Almaguer et al., 2014), undergo severe losses during the brewing process, due to incomplete extraction, adsorption to insoluble malt proteins and to yeast cells during fermentation. Furthermore, XN and DMX readily isomerize (cyclize) to the flavanones isoxanthohumol, and to a mixture of 6- and 8-prenylnaringenin, respectively, in the boiling wort, the same flavanols being anyway endowed with distinct anticarcinogenic and antioxidant properties (Karabin et al., 2015; Magalhães et al., 2008; Stevens, Taylor, Clawson, & Deinzer, 1999). Consequently, XN contents in beers are usually 1%–10% those of isoxanthohumol, while most beers contain no DMX due to its thermal isomerization in the brew kettle (Stevens & Page, 2004).

Another interesting bioactive compound found in beer is the prenylated flavanone 6-geranylnaringenin (6-GN, molecular formula $C_{25}H_{28}O_5$), also known as Bonannione A. It is found only in traces in hops and is mostly generated in the boiling wort from conversion of 3′-geranylchalconaringenin (3′-GCN, molecular formula $C_{25}H_{28}O_5$) (Stevens, Taylor, Clawson, et al., 1999). 6-GN displays significant antibacterial activity (Wang, Tan, Li, & Li, 2001), while little is known about its effects on cancer cells (Venturelli et al., 2016).

The subject of this study is the investigation about the fate of XN, DMX, and 6-GN, in beers produced by means of a new brewing process based on controlled hydrodynamic cavitation (HC), which replaces the most important and critical steps of beer making, from mixing the water with grains, to the whole mashing and hopping steps (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2017).

HC-assisted beer brewing showed several advantages, without apparent drawbacks, such as increasing efficiency in starch extraction, dramatically speeding up enzymatic saccharification, removing traditional, demanding steps, such as dry milling and boiling, as well as reducing gluten concentration up to the threshold of gluten-free beer (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2017). Additional efficiency gain in brewing up to 100% raw unmalted wheat with exogenous enzymes was proven, along with the retention of valuable bioactive compounds from the brewed cereals (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2018).

Cavitation in liquids occurs whenever the local hydrodynamic pressure falls below the liquid"s vapor pressure at a given temperature, causing vaporization in a myriad of bubbles on the micro- to nano-scale, in turn then imploding under pressure recovery (Carpenter et al., 2017). In such collapse events, temperature and pressure increase dramatically up to 5000–10,000 K and 300 atm, respectively, generating very strong shear forces, micro-jets and pressure shockwaves (Pawar, Mahulkar, Pandit, Roy, & Moholkar, 2017; Yasui, Tuziuti, Sivakumar, & Iida, 2004). The same mechanical and thermal effects were deemed responsible for the inactivation or destruction of spoilage and pathogenic microorganisms in food liquids (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2015; Yusaf & Al-Juboori, 2014), as well as key factors in all the other HC-assisted food liquid processes, including the enhanced extraction of bioactive compounds (Albanese, Ciriminna, Meneguzzo, & Pagliaro, 2017; Carpenter et al., 2017; Li, Chen, Zhang, & Fu, 2017).

Cavitation can be harnessed and controlled by means of different mechanisms, among which mechanical methods are recognized as the most energy efficient, robust, and easily scalable, generating hydrodynamic cavitation by means of liquid acceleration, *e.g.* across nozzles, and the associated pressure drop according to Bernoulli's equation (Gogate & Pandit, 2011).

In the treatment of viscous food liquids, especially with solid particles, the most suitable HC reactors are often Venturi tubes (Zamoum & Kessal, 2015; Šarc, Stepišnik-Perdih, Petkovšek, & Dular, 2017). They mitigate the obstruction risk, offer simplicity and robustness, as well as superiority over other types of reactors, such as orifice plates, in terms of inactivation of spoilage microorganisms (Albanese et al., 2015).

Despite the generation of powerful oxidants, such as hydroxyl radicals (·OH, oxidation potential 2.80 eV) in collapsing cavitation bubbles with inner temperatures over 2500 K (Ciriminna, Albanese, Meneguzzo, & Pagliaro, 2017; Podbevsek, Colombet, Ledoux, & Ayela, 2018), absent any further advanced oxidation processes, the extent of oxidation of the bulk liquid medium is quite limited (Yusaf & Al-Juboori, 2014). Indeed, no oxidation was observed either in wort or in final beer produced by means of HC-assisted brewing processes (Albanese et al., 2017).

A single dimensionless parameter, *i.e.* the cavitation number (σ) derived from Bernoulli's equation, as per Eq. (1), practically identifies different HC regimes (Yan & Thorpe, 1990; Šarc et al., 2017).

$$\sigma = (P_2 - P_v)/(0.5 \cdot \rho \cdot u^2) \quad (1)$$

where P_0 (Nm^{-2}) is the average pressure downstream a nozzle (*i.e.*, the recovered pressure), P_v (Nm^{-2}) is the liquid vapor pressure at a specific temperature, ρ (kgm^{-3}) is the liquid density, and u (ms^{-1}) is the flow velocity through the nozzle.

Recently, Slovenian scholars raised important issues about the use of the cavitation number (Šarc et al., 2017), producing a comprehensive set of suggestions and recommendations, aimed at improving the understanding and repeatability of HC processes and experiments. They showed that, changing the very definition of the different parameters in Eq. (1), could lead to differences in σ values as large as two orders of magnitude, recommending that pressure P_0 and velocity u are always measured downstream of the cavitation constriction and through it, respectively.

In order to comply with the above-mentioned recommendations, as well as to allow repeatability, the relevant details of the HC device and related sensors are supplied in Section 2.

2. Materials and methods

2.1. Brewing unit

Fig. 1 shows the experimental device, implementing the HC-based innovative beer-brewing technology on the microbrewery scale, including a closed hydraulic loop with total volume capacity around 230 L, powered by a centrifugal pump (7.5 kW nominal mechanical power, rotation speed 2900 rpm), designed to perform the mashing and hopping stages for all the brewing tests (Albanese et al., 2017).

Any surface in contact with the wort was crafted in food-quality stainless steel (AISI 304), with 2 mm minimum thickness. The cavitation reactor, in the form of a circular-section Venturi tube, described in detail in a past study (Albanese et al., 2015), was preferred over an orifice plate due to both obstruction produced by the circulating solid particles, and to the observed superiority for microbiological disinfection.

The circulating liquid (wort) can be exposed to the atmospheric pressure, or to a given additional hydraulic pressure limited by a tunable pressure release valve, by means of which the cavitation number σ can be tuned across a wide range of values through the P_0 term in Eq. (1) (Soyama & Hoshino, 2016).

While circulating, the beer wort heats up, due to the conversion of impeller's mechanical energy into thermal energy; the heating source concentrates just downstream the cavitation reactor, where vigorous internal friction occurs due to the cavitation process.

All the HC tests ran in brew-in-the-bag (BIAB) mode, using the malts caging vessel shown in Fig. 1, while the hops were allowed free circulation around the hydraulic circuit. A comparative brewing test was