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

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\textbf{A B S T R A C T}

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 by processes based on hydrodynamic cavitation, as well as to 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′-trimethoxy-6′-methoxychalcone, hereinafter also denoted as XN, molecular formula C\textsubscript{24}H\textsubscript{22}O\textsubscript{5}) is a prenylated flavonoid secreted by hop (\textit{Humulus lupulus}) inflorescences. XN is unique to hops, the most abundant polyphenol found in hard resins in the hop lupulin glands (Almaguer, Schönberger, Gasti, 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, et al., 2015), anti-atherosclerotic (Hirata et al., 2017), and anti-carcinogenic, both \textit{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...
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, Hudečová, Jelínek, & Dostálek, 2016), as well as, sometimes, aroma and taste (Dresel, Vogt, Dunkel, & Hofmann, 2016). Moreover, unclari

XN and other prenylated hops' flavonoids, such as desmethylxanthohumol (DMX, molecular formula C_{25}H_{28}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-prenylxanthohumins, respectively, in the boiling wort, the same flavanons 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 based on controlled hydrodynamic cavitation (HC), which replaces the most important and critical steps of beer making, from mixing the water.

HC-assisted beer brewing showed several advantages, without apparent drawbacks, 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; Yufas & 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, Stepniček-Perdih, Petkoviček, & 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 (Yufas & 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).

\[ \sigma = \frac{P_2 - P_0}{0.5 \rho u^2} \]  

where \( P_0 \) (Nm^{-2}) is the average pressure downstream a nozzle (i.e., the recovered pressure), \( P_2 \) (Nm^{-2}) is the liquid vapor pressure at a specific temperature, \( \rho \) (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 230L, 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
performed by means of a conventional Braumeister (Ofterdingen, Germany) model 50 L brewer.

2.2. Analytical instruments and methods

2.2.1. Process parameters

The main production unit, shown in Fig. 1, was equipped with standard thermometer and manometer sensors. In particular, the wort pressure \( P_0 \), contributing to the cavitation number in Eq. (1), was measured far downstream the nozzle of the Venturi reactor, as shown by the location of the pressure gauge, thus complying with the recommendations mentioned in Section 1 (Šarc et al., 2017). The power and electricly consumed by the HC-assisted process were measured by means of a commercial three-phase digital power meter, which was also used to assess the flow velocity through the nozzle of the cavitation reactor, according to the procedure explained in a past study (Albanese et al., 2017), as well as again complying with the aforementioned recommendations.

2.2.2. Basic beer parameters

Physico-chemical and physiological parameters of beers were measured by means of a 6-channel photometric device (CDR, Firenze, Italy, model BeerLab Touch). In particular, alcohol content (0–10% in volume, resolution 0.1%), bitterness on the International Bitterness Unit (IBU) scale (5–100, resolution 0.1), color on the European Brewery Convention (EBC) scale and on the Standard Reference Method (SRM) scale. All reagents were of analytical grade.

2.2.3. Measurement of hops prenylflavonoids

Liquid chromatography coupled to mass spectrometry, a well-established methodology for food analysis (Di Stefano et al., 2012), was used to assess the amount of XN, DMX, and 6-GN, in four different beer samples.

The beer samples were filtered over PTFE syringe filter 0.45 μm. The three compounds under study were identified by ultra-high-performance liquid chromatography, heated electrospray, and mass spectrometry (UHPLC-HESI-MS). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromelogen 7.2 Software (Thermo Fisher Scientific, Bremen, Germany), coupled to a photodiode array detector (Thermo Fisher). A UHPLC column (Phenomenex Luna C18 (2) 50 × 1 mm, 2.5 μm) was used for separation of the selected compounds at 30 °C. The mobile phases used were 0.1% formic acid in water (A) and ACN with 0.1% formic acid (B). The gradient elution program was: 0–3 min 5% B; 3–30 min linear increase to 95% B, 30–35 min 95% and 35–40 min B returning to the initial conditions until full stabilization. The injection volume was 1 μL, and the flow rate was 50 μL min⁻¹.

MS detection was performed using a Q-Exacte accurate-mass spectrometer (Thermo Scientific, Bremen, Germany). The HESI parameters were set using negative ion mode with spectra acquired over a mass range from \( m/z \) 100–800. The optimum values of HESI-MS parameters were: sheath gas flow at 25 arbitrary units; auxiliary gas unit flow at 6 arbitrary units; capillary temperature at 320 °C; auxiliary gas heater temperature at 150 °C; spray voltage at 3.0 kV; and S lens radio frequency level set at 50.

The automatic gain control was set with a maximum injection time of 200 ms. HESI-MS spectra yield single deprotonated ion, \([M−H]^−\), at the same time as the mode FULL-SCAN and t-SIM (targeted Selected Ion Monitoring), to increase sensitivity. The total UHPLC-HESI-MS method run time was 40 min. Detection of xanthohumol was based on calculated exact mass and on retention time of standard compound. Desmethylxanthohumol and 6-geranylnaringenin were assessed only based on the calculated exact mass with data evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA, USA). The linearity of the MS response was verified with solutions containing xanthohumol standard at seven different concentration levels over the range 0.005–1 ppm.

Xanthohumol (purity ≥ 99%) was purchased by Extrasynthese, France. A stock standard solution was prepared at a concentration of approximately 0.1 mg/mL in 80:20 MeOH/H₂O (v/v). The other standard solutions (at 1.0, 0.5, 0.2, 0.05, 0.02, 0.01, 0.005 ppm) were prepared by dilution of the stock solution. Calibration curves were constructed by injecting each standard solution at each concentration level in quadruplicate. The peak areas were calculated and plotted against the corresponding concentrations of the standard compound using linear regression (least squares) to generate the standard curve. Content of desmethylxanthohumol and 6-geranylnaringenin in samples were evaluated using xanthohumol as a reference compound.

2.3. Brewing ingredients

Pilsner or Pale malts were used, along with smaller fractions of Cara Pils and Cara Hell. About the hops, different combinations of pelletized German Perle (hereinafter, Perle), Saaz and German Hersbrucker (hereinafter, Hers) were used. Table 1 shows the average concentration of the three prenylflavonoids under consideration in the used hops, drawn from available sources (Dresel et al., 2016; Stevens, Taylor, & Deinzer, 1999).

<table>
<thead>
<tr>
<th>Hop</th>
<th>Xanthohumol (mg/100 g)</th>
<th>Desmethylxanthohumol (mg/100 g)</th>
<th>6-geranylnaringenin (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perle</td>
<td>1150</td>
<td>4.046</td>
<td>0.018</td>
</tr>
<tr>
<td>Hers</td>
<td>386.6</td>
<td>2.522</td>
<td>0.018</td>
</tr>
<tr>
<td>Saaz</td>
<td>1053</td>
<td>0.674</td>
<td>0.018</td>
</tr>
</tbody>
</table>

a Source: (Dresel et al., 2016).

b Source: (Stevens, Taylor, & Deinzer, 1999). Concentration of 6-GN is extremely low in hops and differences between hop’s types are unknown, it being generated in the boiling wort from conversion of hops’ geranylchalcone 3’-geranylchalconarinigenin.

c Hers = Hallertau Hersbrucker hop.
Fermentation was activated by means of the dry yeast strain Safale US-05, requiring temperature between 15 °C and 24 °C and maximum alcohol content 9.2%, used in any test in the identical proportion of 67 g per 100 L.

### 2.4. Brewing tests

Table 2 summarizes few basic features of the brewing tests, labeled B1, C6, C7 and C10. As mentioned in subsection 2.1, one of the tests (B1) was performed by means of the conventional Braumeister model 50 L brewer.

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Production unit</th>
<th>Net volume (L)</th>
<th>Malts</th>
<th>Hops</th>
<th>Fermentation</th>
<th>Beer color(^c) (EBC/SRM)</th>
<th>Alcohol(^d) (%vol)</th>
<th>IBU(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>B-50</td>
<td>50</td>
<td>Pilsner 6.25 kg</td>
<td>Perle 0.025 kg</td>
<td>Standard</td>
<td>6/3.2</td>
<td>3.2</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Pils 0.9 kg</td>
<td>Hers 0.075 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Hel 0.65 kg</td>
<td>Saaz 0.05 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>HC</td>
<td>170</td>
<td>Pilsner 25 kg</td>
<td>Perle 0.3 kg</td>
<td>Standard</td>
<td>6/2.8</td>
<td>5.0</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Pils 3.6 kg</td>
<td>Hers 0.2 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Hel 2.6 kg</td>
<td>Saaz 0.4 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>HC</td>
<td>170</td>
<td>Pilsner 28.5 kg</td>
<td>Hers 0.6 kg</td>
<td>Standard</td>
<td>5/2.7</td>
<td>3.4</td>
<td>30.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Pils 2.5 kg</td>
<td>Saaz 0.5 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>HC</td>
<td>170</td>
<td>Pale 26 kg</td>
<td>Perle 0.2 kg</td>
<td>Installation</td>
<td>5/2.5</td>
<td>3.9</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cara Pils 3 kg</td>
<td>Hers 0.1 kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) B-50 = Braumeister model 50-L; \(^b\) HC = experimental installation shown in Fig. 1 – variant with malts caged in a cylindrical vessel (BIAB).

### 3. Results and discussion

Fig. 3(a–c) show the amounts of XN, DMX and 6-GN found in the analyzed beer samples, compared with published concentrations of XN and 6-GN for Ale-style beers (Rothwell et al., 2013), while no values are available for DMX due to its general absence from final beers (Stevens & Page, 2004).

The “conventional” beer B1 contained neither XN, nor 6-GN, but only DMX at the level of 37 μg/L. The absence of XN occurs despite the insertion of more than 80% of hops within 16 min before the end of the wort-boiling stage, which should have limited its cyclization to iso-xanthohumol. However, that time could be not enough for the extraction of XN in the boiling wort. The small but measurable value of DMX could derive from the very late insertion of most hops (6 min before the end of the boiling stage), as shown in Fig. 2(a), under the hypothesis that the extraction of DMX is faster than for XN.

Sample C6, obtained from the test including the longest cavitation process, most of which carried out after mashing-out, as shown in Fig. 2(b), also contains no XN. That beer shows a concentration of DMX comparable to sample B1 (39 vs. 37 μg/L), but affected by a much larger uncertainty, as well as a measurable concentration of 6-GN (29 μg/L). Since the latter is mostly generated in the boiling wort from conversion of the 3′-GCN geranylchalcone found in the hops (see Section 1), the HC process appears generating more 6-GN than conventional wort boiling.

Sample C10 does contain XN (93 μg/L), as well as its DMX concentration almost doubles to 76 μg/L in comparison with sample C6, and a significant 6-GN concentration is also observed (38 μg/L). In the respective test, the last hop insertion (Hers, 100 g) was performed 17 min before the end of the warming phase, undergoing temperatures between 99 and 101 °C. Subsequently, after yeast pitching, hops underwent a long cavitation process (about 4 h), at low temperatures (about 27 °C), as shown in Fig. 2(d). Therefore, low temperature HC processes could reveal ineffective for the conversion of DMX into a mixture of 6- and 8-prenylavonoids.

A dramatic increase in the concentration of all three prenyl-avonoids is observed in the beer sample C7, i.e. XN, DMX and 6-GN in the amounts of 127 μg/L, 148 μg/L, and 55 μg/L, respectively. In particular, the high DMX concentration can hardly be explained, because the second and last insertion of Hers hop (400 g) occurred 41 min before the end of the respective HC-assisted brewing process, at temperatures of 95–100 °C, as shown in Fig. 2(c).

About the comparison with values typical of Ale-style beers, Fig. 3(a) shows that XN concentrations in samples C7 and C10 fall into the range of published values, with the former in the upper range, while Fig. 3(b) shows that DMX concentrations are quite surprising, as DMX is hardly found in beers (see Section 1).

In sum, under certain conditions, HC-assisted brewing allows retaining the most important hops' prenylflavonoid, i.e. xanthohumol, in
final beers, at concentrations common to conventionally produced beers, but much greater than in the performed conventional test (actually, zero). As well, under the same conditions, HC-assisted brewing is able to retain desmethylxanthohumol, another hops' prenylflavonoid, significantly better than conventional brewing processes.

The common behavior for XN and DMX is hardly surprising, based on the common losses and conversion mechanisms mentioned in Section 1, i.e. incomplete extraction from hops in the wort, adsorption to insoluble malt proteins, and adsorption to yeast cells during fermentation, besides isomerization in the boiling wort.

The hypothesis can be advanced, that HC-assisted brewing helps retaining XN and DMX due to the absence of the boiling stage, thus reduced isomerization, the effective extraction from hops – already proved for hops' α-acids (Albanese et al., 2017) – as well as, likely, the enhanced solubilization of certain malt proteins (Albanese et al., 2017).

Fig. 3(c) deserves a separate discussion, because the concentrations of 6-GN in all the samples, produced by means of HC-assisted brewing processes (C6, C7, and C10), exceed the respective concentration range found in Ale beers. The measured level in sample C7 falls in the upper range of the substantially higher values found in Dark beers (Rothwell et al., 2013; Stevens, Taylor, & Deinzer, 1999). Along with the absence of measurable 6-GN in the conventional sample B1, the above observations suggest that, under certain conditions, HC is quite effective in extracting 3'-GCN from hops, and/or in generating 6-GN in the processed wort.

Fig. 4(a–c) show the concentrations of the considered prenylflavonoids in the beer samples, along with the respective concentrations in the wort for all the tests, the latter deriving from the inserted hops as per Table 2, and based on hops' data listed in Table 1.

Fig. 4(a) shows that in sample C7, with the greatest absolute XN concentration, the latter is 0.3% of its input value, while sample C10 retains by far the greatest amount of XN, i.e. 0.6%. Based on the comparison with results from the other tests, and the respective timing and conditions of hops insertion, the conclusion can be drawn that HC enhances XN extraction from hops, and likely reduces its adsorption to insoluble malt proteins, conditioned upon short residence time of hops during cavitation at high temperature levels. In particular, based on the available data, hops' residence times at temperatures above about 80 °C, during cavitation, should be less than about 40 min, possibly around 15–20 min, while a lower limit on time residence cannot be inferred. At much lower temperatures, i.e. less than 30 °C as in test C10 (Fig. 2(d)), cavitation does not seem to significantly affect XN concentrations. Therefore, HC-assisted brewing can help producing beers higher in XN concentration, conditioned upon inserting hops with higher XN specific content (such as Perle or Saaz, as per Table 1) as late as possible in the hot wort, i.e. just in time for achieving the desired level of iso-α-acids concentration (Albanese et al., 2017). Moreover, the above results suggest that HC-assisted brewing could help the efforts to increase the XN concentration in final beers, such as by means of XN-enriched hops extracts, due to the likely partial solubilization of malt proteins.

A more surprising pattern arises from Fig. 4(b). Both beer samples C7 and C10 show DMX concentrations higher than the respective input values, at the levels of 136% and 122%, respectively, which could be the result of uncertainties affecting the real DMX concentrations in hops. However, the contrast with sample B1 and sample C6 (57% and 33% of input concentration, respectively) is striking. The same cavitation conditions inferred for superior retaining of XN, appear to work even better with DMX, whose extraction could have been boosted by
the HC processes, and cyclization to 6- and 8-prenylaringenin could have been prevented, even if the specific mechanisms remain unknown. The slightly better results achieved in test C7, compared with test C10, could be due to the greater solubilization of malt proteins in the former, longer test, and/or to the different used malts.

Fig. 4(c) shows that 6-GN concentrations in beers deriving from HC-assisted processes are much greater than the respective input values, i.e. 30 times in sample C6, 47 times in test C7 and as much as 120 times in test C10, confirming that 6-GN results largely from the conversion of other compounds in the processed wort, namely hops’ 3’-GCN (see Section 1).

Aiming at a rough assessment of the conversion rates from hops’ 3’-GCN to beers’ 6-GN, the average content of 1% of total resin flavonoids for several hops varieties has been assumed for 3’-GCN, and compared with the average content of 87% for XN (Stevens, Ivancic, Hsu, & Deinzer, 1997). Therefore, the concentration of 3’-GCN for each hop variety used in the tests was assumed as 1.5% of the respective XN concentration as per Table 1.

Fig. 5 shows the concentrations of 6-GN, along with the initial concentrations of 3’-GCN in the wort.

The ratio of 6-GN to input 3’-GCN concentration in beer samples change from 0% in the conventional test B1, to 5.1% in C6, 10.7% in C7, up to 20.9% in C10, which, along with the absence of 6-GN in sample B1, and the much higher values in samples C6, C7, and C10, in comparison with Ale beers (Fig. 3(c)), suggests that HC likely allows a far more effective extraction of the 3’-GCN geranylchalcone from hops, in comparison to traditional brewing processes.

Finally, the relative superiority of test C10 suggests that the same operational conditions, retaining more of XN and DMX, can apply to boost the generation rate of 6-GN in the processed wort. Such conditions dictate short processing times for hops at high temperatures, which is also beneficial for energy saving.

These promising results could stimulate further research on the fate of bioactive products from hops to beer; in particular, the analysis of the transformation and resulting concentrations of XN, DMX and 6-GN compounds, under a much broader spectrum of HC-assisted brewing ingredients and conditions.

Open research issues in the frame of HC-assisted brewing concern the viability of XN-enrichment of beers, the transformation of other, minor constituents in hops’ resins, as well as the respective impact on beer quality, shelf life, and healthy properties.

The findings of this study agree with recent literature, showing HC viability, efficiency, effectiveness and scalability to boost the extraction of food bioactive compounds (Roohinejad et al., 2016), as well as to release bound phenolics, and increase the antioxidant activity of processed food (Albanese et al., 2018; Lohani, Muthukumarappan, & Meletharayil, 2016; Martynenko & Chen, 2016).

4. Conclusions

HC-assisted brewing was proven able to retain, or generate, higher concentration of certain important and unique hops’ bioactive compounds in the final beer, such as the prenylfлавonoids xanthohumol, desmethylxanthohumol, and 6-geranylnaringenin, thus offering a new, viable way to produce healthier beers, while, potentially, contributing to the beers shelf life.

This capability adds to other proven general advantages, such as cheaper, more efficient, safe and reliable, and more environmentally friendly processes (Albanese et al., 2017), as well as gluten reduction without additives or complex technological processes (Albanese et al., 2017), and extremely efficient brewing of up to 100% raw unmalted wheat (Albanese et al., 2018).
Optimal cavitational and process conditions, aimed at retaining and generating the hops’ bioactive compounds under consideration, were preliminarily identified, even if much research remains to be done, exploring a wider range of beer recipes, and fully exploiting the promising capabilities of HC-assisted brewing, including the use of different cavitators.

Declaration of interest

L.A. and F.M. were appointed as Inventors in the patent submitted on August 9, 2016, international application No. PCT/IT/2016/000194 “A method and relative apparatus for the production of beer”, pending.

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