Radical Scavenging Capacity of hop-derived Products

Dietary antioxidants are believed to be effective in the prevention of oxidative stress related diseases (e.g., cancer and cardio-vascular diseases). Polyphenols are widely recognized as potent antioxidants as they can scavenge reactive oxygen species (ROS). The hop plant (Humulus lupulus L.), used in a variety of health applications and indispensible as a beer ingredient, is an interesting source of polyphenolic antioxidants including tannins, flavonol glycosides and prenylated flavonoids. In addition, also hop oil and hop acids (including downstream products) have been reported as potent antioxidants. In this investigation, the radical scavenging activity of hop products (including different extracts and downstream products) was investigated using two different antioxidant assays: the ORAC to study the peroxy radical scavenging capacity and HORAC to investigate the hydroxyl radical scavenging capacity. Quercetin and a grape extract containing oligomeric proanthocyanidins (OPC) were used for comparison. The peroxy radical scavenging capacities of prenylated flavonoids were highly analogous to quercetin and OPC equaling 5–10 Trolox equivalents. The hydroxyl radical scavenging capacities of Xantho-Flav™ products correlated with the concentration of xanthohumol and pure xanthohumol (> 95 %) corresponded to about 60 Trolox equivalents, which is 10–20 times higher than that of quercetin and OPC. Consistently, ethanol extracts showed a higher radical scavenging activity than CO2-extracts. Furthermore, tannin extract proved an efficient peroxy radical scavenger. Thus it can be concluded that xanthohumol-containing products show high radical scavenging capacities, which partly may be mediated by its metal ion chelating properties. Apart from their possible health benefits, these products might also contribute antioxidant power during the brewing process and during storage.

1 Introduction

It is well-known that a healthy diet, including a lot of fruit and vegetables, is a major determinant for an overall healthy life and prevention of diseases. Already more than 2400 years ago, the Greek philosopher Hippocrates stated: ‘Let food be thy medicine and medicine thy food’. We now know that fruits and vegetables are excellent sources of dietary antioxidants, that can interfere with oxidative processes and may help in the prevention of oxidative stress-related diseases, such as cancer and cardiovascular diseases. Polyphenols have been widely investigated as a most important class of potent antioxidants as they can readily scavenge reactive oxygen species (ROS) [1, 2].

Hops, the typical female organs of the hop plant (Humulus lupulus L.), are rich in secondary metabolites, most of which are secreted in the specialized lupulin glands (hop acids, hop essential oil, prenylated flavonoids) (see Fig. 1). Hops are indispensible as a beer ingredient, as they impart flavour (bitterness and hoppy flavour), enhanced foam stability and bacteriostatic activity. In addition, hop-derived products are used in a variety of traditional and novel health applications (as sleeping aid [3], or for its estrogenic [4] and anti-inflammatory properties [5]). It should be noticed that hops are also an excellent source of polyphenolic antioxidants including proanthocyanidins, flavanols, phenolic acids, stilbenes, flavonols, multifidols and prenylated flavonoids. In addition, also hop oil and hop acids (including downstream products) have been reported as potent antioxidants [6, 7]. Antioxidants can also confer technical advantages, such as increased flavour stability and extended shelf life. In this investigation, we evaluated the antioxidant capacities of different hop products by using two assays (ORAC and HORAC assay) based on different antioxidant mechanisms.

2 Materials and methods

2.1 Hop products

A range of hop products (including different extracts – including different Xantho-Flav™ extracts covering a broad range of concentrations of xanthohumol – and downstream products, see Table 1) was provided by Hopsteiner (Mainburg, Germany). Product specifications can be found on www.hopsteiner.com.
2.2 Chemicals

Fluorescein sodium salt, (±)-6-hydroxy-2,5,7,8-tetramethylchro-
mane-2-carboxylic acid, hydrogen peroxide 30 %, and copper
(II) sulphate 98 %, were purchased from Sigma-Aldrich (Bornem,
Belgium). Random methylated β-cyclodextrin (RMCD), DS-12
was purchased from Cyclolab (Budapest, Hungary).

2.3 Hydrophilic ORAC assay

The ORAC-assay was an adaptation of the protocols proposed by
Ou et al. [8]. Sodium fluorescein was dissolved in phosphate buffer
solution (PBS) (75 mM, pH = 7.0) to obtain a stock solution of
4.8 mM. The working solution (48 mM) was obtained by subsequent
dilution in PBS. A 10 ml solution of AAPH (2,2'-azo-bis(2-amid
iminopropane)dihydrochloride) was prepared at a concentration
of 64 mM. For each session of measurements, a standard curve of
Trolox was plotted (3–40 µM). Trolox solutions were prepared in
PBS. All measurements were performed in triplicate. A blank was
run with each assay. The analysis was performed using black 96-well
microwell plates (NuncTM) and a multilabel counter (Perkin Elmer).
Sample (25 µl) was mixed with sodium fluorescein (150 µl) and
incubated for 10 min at 37 °C. AAPH (25 µl) solution was then added
to the mixture and the microplate was shaken. The fluorescence
(λexcitation = 485/14 nm; λemission = 535/25 nm) was registered every
minute for one hour. The quantitation of the antioxidant capacity
was based on the calculation of the area under the curve (AUC).

2.4 Lipophilic ORAC assay

For the hop oils and non-water soluble products, the lipophilic
variant of ORAC was used according to the procedure described by
Hu et al. [9]. A 48 mM working solution of sodium fluoresce-
in and a 320 mM solution of AAPH in PBS was used. For each
session of measurements, a standard curve of Trolox was plotted
(2–25 µM). Samples and Trolox solutions were prepared in a 7 %
RMCD solution in an acetone/water (1/1, v/v) mixture. The
analysis was performed using microplates (96-well, black, NuncTM)
and a multilabel counter (Perkin Elmer). Sample (20 µl) was mixed
with sodium fluorescein (150 µl) and incubated for 10 sec before every
measurement. The quantitation of the antioxidant capacity
was based on the calculation of the area under the curve (AUC).

2.5 Hydrophilic HORAC assay

The hydroxyl radical scavenging capacity was determined with the
HORAC assay according to the protocol proposed by Ou et al. [8]
and Gerhäuser et al. [10]. Sodium fluorescein was dissolved in
PBS (75 mM, pH = 7.0) to obtain a stock solution. The working
solution (48 mM) was obtained by subsequent dilution in PBS.
The 30 % H2O2 solution was diluted with distilled water to a 12 %
solution. A 360 µM working solution of copper sulphate was made.
For each session of measurements, a standard curve of Trolox was
plotted (25–300 µM). Trolox solutions were prepared in PBS. All
measurements were performed in triplicate. A blank was run with
each assay. The analysis was performed using microplates (96-well,
black, NuncTM) and a multilabel counter (Perkin Elmer). After adding
20 µl H2O2 solution and 100 µl of sodium fluorescein solution to
25 µl of sample the microplate was shaken. Copper sulphate
was then added and the microplate was shaken. The fluorescence
(λexcitation = 485/14 nm; λemission = 535/25 nm) was registered every
minute for one hour. The microplate was shaken for 10 sec before
every measurement. The quantitation of the antioxidant capacity
was based on the calculation of the AUC. The hydrogen radical
scavenging capacity was expressed as µmol of trolox equivalents
(TE) per gram of tested product.

2.6 Lipophilic HORAC assay

For the hop oils and non-water soluble products, a lipophilic
variant of HORAC was used, including RMCD as a solubility
enhancer. Sodium fluorescein was dissolved in PBS (75 mM,
pH = 7.0) to get a stock solution. The working solution (48 mM)
was obtained by subsequent dilution in PBS. The 30 % H2O2
solution was diluted with distilled water to obtain a 12 % solution.
A 720 µM working solution of copper sulphate was made. For each
session of measurements, a standard curve of Trolox was plotted
(2–25 µM). Samples and Trolox solutions were prepared in a 7 %
RMCD solution in an acetone/water (1/1, v/v) mixture. The
analysis was performed using microplates (96-well, black, NuncTM)
and a multilabel counter (Perkin Elmer). Samples were mixed
with sodium fluorescein and H2O2. The microplate was shaken
after addition of the copper sulphate solution. The fluorescence
(λexcitation = 485/14 nm; λemission = 535/25 nm) was registered every
minute for one hour. The microplate was shaken for 10 sec before
every measurement. The quantitation of the antioxidant capacity
was based on the calculation of the AUC. The hydroxyl radical
scavenging capacity was expressed as µmol of trolox equivalents
(TE) per milliliter or gram of tested product.

3 Results and discussion

The two assays used in this investigation to assess the antioxidant
capacity are based on different mechanisms of action. The ORAC
(Oxygen Radical Averting Capacity) assay reflects the capacity for
Hydrogen atom transfer is an essential step in the termination of
radical chain reactions involved in lipid oxidation. In the HORAC
(Hydroxyl Radical Averting Capacity) assay, antioxidants can be
assessed for their hydroxyl radical preventing capacity [12].
Transition metals (e.g. Cu(II)) react with hydrogen peroxide to form
hydroxyl radicals via the Fenton reaction. Hydroxyl radicals are
very reactive oxidants involved in the oxidation of DNA, lipids
and proteins. The HORAC and ORAC assay measure two different
but equally important aspects of antioxidant properties, i.e. radical
chain breaking and prevention of radical formation.

The peroxyl radical scavenging capacities of all tested prenylated
flavonoids are highly similar to that of the powerful antioxidant
quercetin, equaling 5–10 Trolox equivalents (Table 1). 8-Prenyl-

naringenin was even slightly more active than both xanthohumol and isoxanthohumol. This is consistent with the fact that poly-

phenols bearing multiple OH substitutions possess very strong antioxidant activities against peroxyl radicals [13, 14]. On the other hand, tetrahydroxanthohumol showed a weaker activity than xanthohumol, suggesting that also the double bond in the prenyl side chain and/or the α,β-unsaturated keto-functionality contribute to the peroxyl radical scavenging activity.

In figure 2 the ORAC values of the different Xantho-Flav™ extracts are depicted against their concentration of xanthohumol (X). The radical scavenging capacities of Xantho-Flav™ extracts were highly correlated with the concentration of xanthohumol (X) ($R^2 = 0.975$), suggesting that xanthohumol is largely responsible for the peroxyl radical scavenging activity of these products.

The hydroxyl radical scavenging capacity of pure xanthohumol was about 25 Trolox equivalents which is 10–20 times higher than that of quercetin (Table 1). In contrast to the linear response observed in the ORAC assay, xanthohumol showed an exponential response in the HORAC assay (Fig. 3). This indicates that xanthohumol exerts its hydroxyl radical scavenging activity via one or more different mechanism(s) compared to Trolox, most likely involving chelation of metal ions. A study of Cheng and Breen revealed that transition metal-induced oxidation is strongly inhibited by flavonoids with 3',4'-catechol, 4-oxo, and 5-OH arrangements, like quercetin [15]. As xanthohumol does not contain a catechol group, other functional features, such as the α,β-unsaturated keto-functionality, are most likely to be involved. This is corroborated by the much weaker activity of tetrahydroxanthohumol, isoxanthohumol and 8-prenylnaringenin, which are all lacking this functionality.

Consistent with the observations on the large contribution of xanthohumol to the overall antioxidant activity of hop products, ethanol extract showed a higher radical scavenging activity than CO$_2$-extract. Also, tannin extract proved to be an efficient hydroxyl radical scavenger. Tannins, which are highly polymerized polyphenols, contain many hydroxyl groups and it is therefore likely that they are very effective antioxidants. The antioxidant activities of hop acids and derived products are generally lower than the polyphenolic products (Table 1) and hop oil did not show any significant antioxidant capacity.

4 Conclusion

Hop-derived polyphenols in general and hop-derived prenylated flavonoids in particular are powerful antioxidants able to scavenge peroxyl radicals and to prevent formation of hydroxyl radicals. Xanthohumol, the predominant prenylflavonoid in hops and now available as concentrated extracts (Xantho-Flav™), showed exceptionally high hydroxyl radical averting capacities, which may be partly mediated by metal ion chelating properties. Interestingly, enrichment of xanthohumol in the brewing process has been investigated and has been shown feasible [16, 17]. Apart from their possible health benefits, these products might also contribute antioxidant power during the brewing process and during storage of products containing these compounds.

5 References

15. Cheng, I. F. and Breen, K.: On the Ability of Four Flavonoids, Bai-
cilein, Luteolin, Naringenin, and Quercetin, to Suppress the Fenton
Reaction of the Iron-ATP Complex, Biometals, 13 (2000), no. 1,
pp. 77-83.

in Stout/Porter Beer, Brauwelt, 143 (2003), no. 50, pp. 1709-1712.

17. Wunderlich, S.; Zurcher, A.; Back, W.: Enrichment of Xanthohumol
in the Brewing Process, Molecular Nutrition & Food Research 49
(2005), no. 9, pp. 874-881.

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Appendix

Table 1 Antioxidant capacity of hop-derived products in ORAC and HORAC assays

<table>
<thead>
<tr>
<th>Class</th>
<th>Material</th>
<th>ORAC</th>
<th>HORAC</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Antioxidant</td>
<td>Molar antioxidant</td>
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<tr>
<td></td>
<td></td>
<td>capacity</td>
<td>equivalents</td>
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<tr>
<td></td>
<td></td>
<td>(µmol Trolox/g)</td>
<td>(vs Trolox)</td>
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<tr>
<td>References for comparison</td>
<td>Quercetin-dihydrate (90 %)</td>
<td>21779</td>
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<tr>
<td></td>
<td>Grape OPC extract</td>
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<tr>
<td>Purified Compounds (&gt; 98 %)</td>
<td>Isoxanthohumol</td>
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<td></td>
<td>8-Prenylnaringenin</td>
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<td></td>
<td>Xanthohumol</td>
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<td></td>
<td>Tetrahydroxanthohumol</td>
<td>13559</td>
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<td>Xantho-Flav (at 15 % X)</td>
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<td>Xantho-Flav (at 40 % X)</td>
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<td>Xantho-Flav (at 90 % X)</td>
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<td>Other Hop Products</td>
<td>Ethanol extract (Hallertau Taurus)</td>
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<td>Carbon dioxide extract (Hallertau Taurus)</td>
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<td>Tannin Extract (liquid – 45–50 % water)</td>
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<td>Tannin Extract (dried)</td>
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<td>Hop oil (Type Dry)</td>
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<td>Carbon dioxide extract (Apollo)</td>
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<td>α-acids (10 % in PG)</td>
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<td>β-acids (40–50 % in Beta Aroma Aroma Extract)</td>
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<td>PIKE (Potassium-form Isomerized Kettle Extract)</td>
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<td>Iso extract (30 %)</td>
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<td>Tetra concentrate (65–70 %)</td>
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Fig. 1  Hop-associated secondary metabolites

Fig. 2  Peroxyl scavenging capacity of Xantho-Flav™ extracts as assessed in the ORAC assay

Fig. 3  Hydroxyl radical scavenging capacity of Xantho-Flav™ extracts as assessed in the HORAC assay