CHEMICAL, BIOLOGICAL AND SENSORY CHARACTERIZATION OF HOPS AND DRY-HOPPED BEERS: PERSPECTIVES FOR THE USE OF PORTUGUESE GENOTYPES

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Introduction

- Aroma and bitterness to beer
- More than 270 varieties
- Aroma, bitter and dual purpose
- ↑ use in the new trend of brewing industry (craft beer)
- ↑ development of new varieties (aroma)
- ↑ use in dry-hopping (aroma)

Figure 1. Hops composition.
# Goals of the study

<table>
<thead>
<tr>
<th><strong>Characterization/ Discrimination</strong>&lt;br&gt;<strong>Portuguese vs Commercial</strong></th>
<th>1. Genotype the population</th>
</tr>
</thead>
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<td>2. Evaluate the suitability of NIR and MIR to discriminate hops (potential authenticity tool)</td>
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<td>3. Evaluate the volatile profile and aroma attributes</td>
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<td><strong>Impact of hops in beer characteristics</strong></td>
<td>4. Evaluate the potential of Portuguese hops genotypes in beer production (sensory impression and odour-active volatile composition)</td>
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<td>5. Predict the extraction of volatile compounds, and the impact on specific sensory characteristics</td>
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<td><strong>Impact of hops in beer bioactivity</strong></td>
<td>6. Optimize and predict the extraction of α-acids and xanthohumol to reach the highest yield</td>
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<td></td>
<td>7. Evaluate, <em>in vitro</em>, the antiproliferative activity of xanthohumol, isoxanthohumol, α and β-acids, and iso-α-acids on colon Caco-2 cells</td>
</tr>
</tbody>
</table>
Sampling

120 Portuguese native hops
97 samples (PTG)
Portuguese Germplasm Bank

23 samples (PTW)
Wild hops

33 commercial

33 reference hops (IHGC codes)
Slovenian Institute of Hop Research and Brewing (Žalec, Slovenia)

Genetic evaluation
leaves

Chemical and sensorial analyses
cones and pellets

Bioactivity assays
pure compounds and pellets

Figure 2. Original places of the collection of Portuguese Germplasm.
## Methodologies

<table>
<thead>
<tr>
<th>DNA HRMA</th>
<th>Characterization/Discrimination</th>
<th>Impact of hops in beer characteristics</th>
<th>Impact of hops in beer bioactivity</th>
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</thead>
<tbody>
<tr>
<td>Genotyping 7 Single Nucleotide Polymorphic (SNP) markers</td>
<td></td>
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</tbody>
</table>

| NIR/ MIR                     | Spectroscopic of vibrational bonds |                                        |                                   |

| HS-SPME-GC-MS                | Volatile profile                  | Olfactometry                             |                                   |
|-------------------------------|---------------------------------|----------------------------------------|                                   |
|                               |                                 | Volatile quantification                 |                                   |

| HPLC                         | α-acids, β-acids, xanthohumol     | α-acids, β-acids, xanthohumol, iso-α-acids, isoxanthohumol |                                   |

| Steam distillation           | Total oil                        |                                        |                                   |

| CATA                         | Sensory analysis                 |                                        |                                   |

| QDA                          |                                 |                                        |                                   |

| Cell culture                 |                                 |                                        |                                   |

|                               |                                 |                                        | Antiproliferative activities      |
1. Genotype the population

- Most of the 143 genotypes analysed were discriminated by 7 SNP markers
- Specificity of the genetic pool of Portuguese wild genotypes

**Table 1. Percentage in cluster**

<table>
<thead>
<tr>
<th>Clade</th>
<th>Portuguese</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>(6) 25%</td>
<td>(18) 75%</td>
</tr>
<tr>
<td>Blue</td>
<td>(15) 54%</td>
<td>(13) 46%</td>
</tr>
<tr>
<td>Black</td>
<td>(89) 98%</td>
<td>(2) 2%</td>
</tr>
</tbody>
</table>

(amount of genotypes) and percentages in clade
2. Evaluate the suitability of NIR and MIR to discriminate hops

<table>
<thead>
<tr>
<th>Infrared methods</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>➢ vibrational spectroscopic*</td>
<td>➢ 33 commercial varieties</td>
</tr>
<tr>
<td>➢ rapid</td>
<td>➢ 5 samples of each</td>
</tr>
<tr>
<td>➢ low-cost</td>
<td></td>
</tr>
<tr>
<td>➢ non-destructive</td>
<td></td>
</tr>
<tr>
<td>➢ environmentally friendly</td>
<td></td>
</tr>
<tr>
<td>(do not generate residues)</td>
<td></td>
</tr>
</tbody>
</table>

* absorbance of molecular vibrations

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Infrared methods:
- vibrational spectroscopic
- rapid
- low-cost
- non-destructive
- environmentally friendly (do not generate residues)
2. Evaluate the suitability of NIR and MIR to discriminate hops

Optimization (best spectral region)
- NIR data between 6,079 and 5,389 cm\(^{-1}\)
- MIR data between 1,880 to 600 cm\(^{-1}\)

Correct prediction
- NIR: 96.6%
- MIR: 94.2%

Figure 4. Hops raw spectra.
Figure 5. Scores of the first two components using best spectral regions.
2. Evaluate the suitability of NIR and MIR to discriminate hops

- NIR chemical characterization provided the discrimination of Portuguese and commercial hops
  - 89.9% of correct prediction

- Grouped in 3 different clusters:
  - 36 Portuguese + TET, EKG, GOL, FUG (α-acids < 6%)
  - 35 Portuguese + 10 commercial (α-acids < 6%)
  - 5 Portuguese + 19 commercial (α-acids > 6%) (PTG38, PTG53, PTG62, PTG63, and PTW7)

Figure 6. Hierarchical cluster analysis using NIR data between 6,079 and 5,389 cm⁻¹.
3. Evaluate the volatile profile and aroma attributes

- Portuguese discriminated from commercial by volatile profile

- Hops: 4 groups with Portuguese hops
  - ✓ 70 hops
  - ✓ PTW7, NBR, NUG and CIT
  - ✓ PTG40 and PTG62
  - ✓ PTG41 and PTG51

- Compounds:
  - → 12 compounds can explain the discrimination

Figure 7. heatmap of 32 odour-active compounds.
3. Evaluate the volatile profile and aroma attributes

- Portuguese hops were discriminated from commercial by sensory characteristic
  - ✓ In general, Portuguese samples were more resinous, spicy and herbal
  - ✓ There were some Portuguese native hops with fruity/sweet, floral, and citrus characteristics

Figure 8. Correspondence analysis of hop samples in the aroma regions selected for check-all-that-apply (CATA).
4. Potential of Portuguese hops genotypes in beer production

Selection of 4 Portuguese hops

- No dry-hopped
- Dry-hopped 3 g/L; 6 days

Base beer: Munich Helles

- Impact on sensory and analytical profile of dry-hopped beers
  - Sensory analysis
  - Volatile quantification
4. Potential of Portuguese hops genotypes in beer production

Selection of Portuguese hops:
- 3 samples presented the best compromise among higher content of the quantified variables
- 1 sample represented the majority (others)

Table 2. α/β-acids, XN, and total oil of Portuguese hops

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acids (%)</td>
<td>1.2</td>
<td>PTW7 = 10.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTW8 = 5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others ≤ 3</td>
</tr>
<tr>
<td>β-acids (%)</td>
<td>1.7</td>
<td>PTW2 = 6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others 0.4 to 4.3</td>
</tr>
<tr>
<td>Xanthohumol (XN) (%)</td>
<td>0.2</td>
<td>up to 0.8</td>
</tr>
<tr>
<td>Total oil (mL/100g)</td>
<td>0.71</td>
<td>0.23 to 1.84</td>
</tr>
</tbody>
</table>
4. Potential of Portuguese hops genotypes in beer production

- Selected Portuguese hops promoted total hoppy, citrus, sweet fruits and spicy characteristics to dry-hopped beers

  ✓ PTW2 and PTW7 showed more fruity/citrus

  ✓ PTG22 more spicy

Figure 10. QDA sensory analysis of beers. Performed by panelist trained for evaluation of beer. Certified by the German agricultural society (Deutsche Landwirtschafts-Gesellschaft e.V.).
4. Potential of Portuguese hops genotypes in beer production

- Selected Portuguese hops promoted ↑ of main volatile compounds, some cases, ↑ over threshold values

✓ PTW2 and PTW7 demonstrated the highest increases

Figure 11. quantification (µg/ L) of compounds.
5. Predict the extraction of volatile compounds and the impact on specific sensory characteristics

Is it possible to predict the sensory perception, knowing the volatile composition?

➢ Initial beers (Munich Helles) presented statistical differences in chemical composition and sensory analyses

➢ Volatile quantification

➢ Sensory analysis

✓ Evolution and relationship

Mandarina Bavaria
Dry-hopping: 3 g/ L
1, 3, 6, 10 & 15 days

* Sesquiterpenes
* Monoterpenoids
* Monoterpenes
* Esters

µg/L
5. Predict the extraction of volatile compounds and the impact on specific sensory characteristics

- Maximum extraction rates were achieved earlier in the most kettle hopped beer
- Some compounds reached maximum concentrations earliest
  - esters *(fruity)*
  - monoterpenoid alcohols *(citrus and floral)*

Highest concentration reached after
- **Beer A:** 6 to 10 days of dry-hopping
- **Beer B:** 10 to 15 days of dry-hopping
5. Predict the extraction of volatile compounds and the impact on specific sensory characteristics

- 1 to 3 days of dry-hopping were enough to denote sensorial changes
- After 3 days there were no more significant differences between beers A and B (except citrus after 15 days)

Figure 13. QDA sensory analysis of beers. Performed by panelist trained for evaluation of beer. Certified by the German agricultural society (Deutsche Landwirtschafts-Gesellschaft e.V.)
5. Predict the extraction of volatile compounds and the impact on specific sensory characteristics

- Myrcene, linalool, 2MB2MP, and α-humulene were the main compounds responsible for modeling the relationship with sensory characteristics

✓ It is possible to predict the sensory intensity, using the quantification of these 4 compounds

Figure 14. Volatile compounds ranked by variable importance for the projection (VIP scores) obtained from PLS-R analysis with 95% confidence interval. $Q^2 = 0.68$, $R^2_X = 0.75$ and $R^2_Y = 0.71$. 
6. Optimize/predict the extraction of α-acids and xanthohumol

Dry-hopping
0.7; 1.4 and 2.8 g/ L
21, 10 and 5 days before the end of maturation

Base beer: Pale Ale

CHINOOK
α-acids = 12.5%
xanthohumol = 0.50%

EAST KENT GOLDINGS
α-acids = 4.0%
xanthohumol = 0.29%

TETTNANGER
α-acids = 1.7%
xanthohumol = 0.26%

Quantification
6. Optimize/predict the extraction of $\alpha$-acids and xanthohumol

*Figures 15. Concentrations of $\alpha$-acids and xanthohumol in beers.*

- **$\alpha$-acids in beer**
  - Maturation time = 13.3 days
  - Concentration = 147 mg/L
  - $\approx$ 1.5 g/L of hops with 10%

- **XN in beer**
  - Maturation time = 13.9 days
  - Concentration = 14 mg/L
  - $\approx$ 2.8 g/L of hops with 0.5%
7. Antiproliferative activity of hops compounds

Pure compounds

- Xanthohumol
- Isoxanthohumol
- Iso-α-acids
- α-acids
- β-acids

Non-hopped beer (Pale Ale)

Spiked beers

Cell culture
Antiproliferative activity on Caco-2 cells (colon adenocarcinoma)
7. Antiproliferative activity of hops compounds

Normal content in commercial beers:
- XN up to 10 µg/mL
- iso XN = 0.03 to 4 µg/mL
- α/β-acids = 0.4 to 5 µg/mL
- iso-α-acids = 1.4 to 40 µg/mL

- xanthohumol = no inhibition (0.313 to 20.000 µg/mL)
- isoxanthohumol ≈ 25% inhibition (at 10 and 20 mg/mL)

Figure 16. Log-dose vs. response curve for α/β-acids and iso-α-acids extracts on Caco-2 cell growth after 48 hours exposure.

- ≈ 60% inhibition
  IC₅₀ = 16 µg/mL
  (25 to 80.00 µg/mL)

- ≈ 60% inhibition
  IC₅₀ = 51 µg/mL
  (5.85 to 100.00 µg/mL)
7. Antiproliferative activity of hops compounds

• α/β-acids and iso-α-acids ↑ the antiproliferative activity of non-hopped beer matrix

Figure 17. Effect of α/β acids and iso-α acids spiked in 100% beer matrix on Caco-2 cell growth after 48 hours exposure.
**Overall conclusions**

✓ Regarding discrimination and identification of hop varieties, the genetic (SNP/ HRMA) and spectroscopic (NIR and MIR) approaches proved to be valuable tools.

✓ Successful models were obtained:

✓ to predict specific sensory perceptions by quantifying of a minimal set of volatile compounds.

✓ to explain maximum efficiency of xanthohumol and α-acids extraction.

✓ Concerning bioactivity:

✓ isoxanthohumol, α/β-acids and iso-α-acids showed significant inhibitory effect in Caco-2 cells proliferation.

✓ an increase in the effect was observed when pure compounds of α/β-acids and iso-α-acids were added to beer matrix.
Overall conclusions

✓ Genetic, chemical and sensory differences were observed between commercial and Portuguese native hops.

✓ Native hops presented low α-acids content, variable amounts of total oil, and different volatile profile.

✓ Hops were more resinous, spicy and herbal and less citrus, fruity/sweet, and floral than commercial varieties. Nevertheless, wide sensory characteristics were described.

✓ Some native hops are promising to be explored by the brewing industry, directly used in beer production or as a source of breeding material for the development of new varieties.
Thank you for being part of this journey!!!