Effect of Barley Variety and Location on the Sensory, Physical and Chemical Characteristics of Lager Beer

Michel Aliani
Michel.Aliani@Umanitoba.ca
Food and Human Nutritional Sciences
Saint Boniface Hospital Research Center
The Canadian Malting Barley Technical Centre
Introduction

• Canada is the world’s leading supplier of high-quality, value-added malting barley and malt products including beers.

• Predicting the sensory outcome of beers produced from new barley varieties is critical to ensure the consumer acceptability (key to successful expansion of the breeding program).

• Development of scientific models to predict sensory outcome based on physicochemical data may be of use to barley breeders and the beer industry.

The main objective of the present study was:

✓ To develop a predictive model to combine the sensory data of lager beer to its physicochemical properties
Flavour Perception

- Olfactory bulb
- Olfactory cells
- Aroma molecules
Materials & Methods

Beer Preparation

• Beer samples were prepared by the Canadian Malting and Brewing Technical Centre (CMBTC), Winnipeg MB.

• Four Canadian malting barley varieties were used:
  – CDC Bow, AAC Connect, CDC Copeland, and Harrington.

• All varieties were grown in 3 locations:
  – Brandon, Lacombe, and Saskatoon.

• All samples were prepared according to standard procedures for lager beer and kept in kegs in refrigerated storage (2oC) for approximately 2 weeks before sampling.
Sensory Evaluation

Recruitment

• Volunteers (n=10) were recruited from the staff at the CMBTC and others working in the same building.

• The only criteria were that volunteers were not allergic to any food or beverage products and that they be available and interested in the study. An honorarium was provided for their participation.

Sample Preparation

• Beers were removed from the refrigerator (2°C) 30 minutes prior to the start of the session, poured down the side of the coded clear plastic beer glass (210 mL) so that foaming occurred but not in excess (50 ± 1 g).

• Odor-free cardboard covers (8 cm diameter) were placed immediately on top of the glasses to allow volatiles to build up.

• Beer was evaluated at 10 to 14°C.
Sensory Evaluation (cont’d)

Group Training Discussion Sessions

- **Sessions (n=10)** were conducted to
  - Familiarize panelists with the attribute definitions and their aroma/flavor characteristics in both filtered water and lager beer samples.
  - Gain experience in evaluating lager beers made from different malt types.
- All samples were coded with **3-digit random numbers**.
- Intensities were marked on **line scales with nine points from 0 (none) to 8 (high)**.
- Samples were expectorated and filtered water (20°C) was provided for cleansing the palate before each sample.

Experimental Sessions

- Panelists were seated at a large table with portable partitions between them.
- Beers from one variety from each of the three locations were evaluated at one session. This was repeated the following day.
- Evaluation of the four varieties was completed in 6 weeks based on the processing schedule.
• 255 – Lacombe
• 148 – Lacombe
• 286 – Brandon
• 527 – Brandon
• 872 – Saskatoon
• 907 – Saskatoon
# Attributes Definitions
(Aroma and Flavor\(^1\))

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulphide</td>
<td>Aroma/flavor associated with dimethyl sulphide in solution</td>
<td>Dimethyl sulphide (FlavorActiV, UK)</td>
</tr>
<tr>
<td>Grainy</td>
<td>Aroma/flavor associated with steeped malt kilned at a relatively low temperature</td>
<td>Hot Steep Malt Sensory Evaluation Method (ASBCMOA-Sensory Analysis-14, 2017)</td>
</tr>
<tr>
<td>Malty</td>
<td>Aroma/flavor associated with steeped malt kilned at an increased temperature</td>
<td>Hot Steep Malt Sensory Evaluation Method (ASBCMOA-Sensory Analysis-14, 2017)</td>
</tr>
<tr>
<td>Sweet</td>
<td>Taste associated with sucralose in solution</td>
<td>Sucralose (FlavorActiV, UK)</td>
</tr>
<tr>
<td>Bitter</td>
<td>Taste associated with iso-alpha-acids in solution</td>
<td>Iso-alpha-acids (FlavorActiV, UK)</td>
</tr>
<tr>
<td>Astringent</td>
<td>Feeling in the mouth associated with drying like that produced by saponins in solution</td>
<td>Saponin (FlavorActiV, UK)</td>
</tr>
</tbody>
</table>

\(^1\) Place the glass in position for sniffing. Remove the cover. Take three short sniffs and replace the cover. Take a sip of the sample. Ensure that the sample thoroughly covers all mouth surfaces. Expectorate the sample.

\(^2\) Evaluate the smell and taste of the sample and mark the intensity using the scale from 0 → 8, where 0 represents no perception of the attribute and 8 represents high intensity. Circle the number which corresponds to the intensity.

\(^3\) All standards thoroughly mixed in lager beer made at CMBTC.
Physical and Chemical Analysis

- Bitterness (IBU)
- pH
- Color (ASBC)
- Specific Gravity
- Apparent Extract (%w/w)
- Alcohol (%v/v)
- Apparent Degree of Fermentation (%w/w)
- Real Degree of Fermentation (%)
- Original Extract (%w/w)
- Real Extract (%w/w)
- Alcohol (%w/w)
- Foam (NIBEM)
- Maltotetraose (mg/L)
- Maltotriose (mg/L)
- Maltose (mg/L)
- Glucose (mg/L)
- Fructose (mg/L)

Non-Volatile Compounds

Water-soluble extraction and untargeted analysis by:

1. Liquid Chromatography–Quadrupole Time of Flight Mass Spectrometry (LC–QTOF–MS (Agilent 1260/6538)). Library with 250,000 compounds.

2. Nuclear Magnetic Resonance (NMR (AVANCE III 600 MHz). Library with 1400 compounds.

Volatile Organic Compounds (VOCs)

- Extraction using a Likens Nickerson Solvent Extraction
  - Gas Chromatography–Mass Spectrometry (GC–MS)
Statistical Analysis

- **2-way ANOVA** was performed for sensory evaluation, beer measurements and NMR compounds. The model included two fixed effects, variety and location, with the interaction.

- **1-way ANOVA** was conducted for volatile measurements with variety as the main fixed effect. Analyses were performed using SAS (2009) software (Statistical Analysis System, Cary, NC).
  - Tukey’s multiple comparison test was used to determine mean treatment differences when significant (p<0.05).

- **Mass Professional Profiler (MPP 12.6, Agilent Ltd.)** was used for Mass spectrometry and NMR results.

- **Partial Least Squares Discrimination Analysis (PLS-R)** was used to determine possible relationships between the sensory attributes and physicochemical measurements from the beer samples throughout production and on the final product (XLSTAT 2016, Addinsoft, Paris, FR).
  - Mean values for all of the variables.
# Sensory Evaluation Results

<table>
<thead>
<tr>
<th>Sensory Attribute</th>
<th>Variety</th>
<th>Location</th>
<th>Variety x Location</th>
<th>AAC Connect</th>
<th>CDC Bow</th>
<th>Copeland</th>
<th>Harrington</th>
<th>Brandon</th>
<th>Lacombe</th>
<th>Saskatoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl Sulfide</td>
<td>0.4 NS</td>
<td>0.2 NS</td>
<td>†</td>
<td>2.5 (1.6)</td>
<td>2.3 (1.6)</td>
<td>2.5 (1.6)</td>
<td>2.5 (1.7)</td>
<td>2.4 (1.6)</td>
<td>2.5 (1.7)</td>
<td>2.4 (1.5)</td>
</tr>
<tr>
<td>Grainy</td>
<td>0.3 NS</td>
<td>0.1 NS</td>
<td>†</td>
<td>2.3 (1.2)</td>
<td>2.4 (1.2)</td>
<td>2.4 (1.4)</td>
<td>2.3 (1.2)</td>
<td>2.3 (1.2)</td>
<td>2.4 (1.3)</td>
<td>2.3 (1.2)</td>
</tr>
<tr>
<td>Malty</td>
<td>0.3 NS</td>
<td>1.6 NS</td>
<td>†</td>
<td>2.0 (1.4)</td>
<td>2.1 (1.2)</td>
<td>2.2 (1.4)</td>
<td>2.0 (1.3)</td>
<td>1.9 (1.3)</td>
<td>2.2 (1.4)</td>
<td>2.1 (1.3)</td>
</tr>
<tr>
<td>Sweet</td>
<td>1.0 NS</td>
<td>0.3 NS</td>
<td>†</td>
<td>1.5 (1.1)</td>
<td>1.6 (1.3)</td>
<td>1.8 (1.2)</td>
<td>1.6 (1.2)</td>
<td>1.7 (1.2)</td>
<td>1.6 (1.2)</td>
<td>1.6 (1.2)</td>
</tr>
<tr>
<td>Bitter</td>
<td>0.9 NS</td>
<td>0.5 NS</td>
<td>†</td>
<td>2.6 (1.2)</td>
<td>2.5 (1.3)</td>
<td>2.7 (1.5)</td>
<td>2.4 (1.3)</td>
<td>2.6 (1.3)</td>
<td>2.5 (1.3)</td>
<td>2.5 (1.3)</td>
</tr>
<tr>
<td>Astringent</td>
<td>1.5 NS</td>
<td>0.4 NS</td>
<td>†</td>
<td>2.6 (1.8)</td>
<td>2.4 (1.5)</td>
<td>2.7 (1.7)</td>
<td>2.3 (1.5)</td>
<td>2.4 (1.6)</td>
<td>2.4 (1.6)</td>
<td>2.6 (1.6)</td>
</tr>
</tbody>
</table>

**NS (Not Significant) probability ≥ 0.05**

† sums of squares for insignificant interaction added to error sums of squares
## Physical and Chemical Analysis

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Variety</th>
<th>Location</th>
<th>Variety x Location</th>
<th>AAC Connect</th>
<th>CDC Bow</th>
<th>Copeland</th>
<th>Harrington</th>
<th>Brandon</th>
<th>Lacombe</th>
<th>Saskatoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Gravity</td>
<td>170.2</td>
<td>171.7</td>
<td>42.8</td>
<td>1.0047 b</td>
<td>1.0035 d</td>
<td>1.0043 c</td>
<td>1.0052 a</td>
<td>1.0047 a</td>
<td>1.0037 b</td>
<td>1.0048 a</td>
</tr>
<tr>
<td>Apparent Extract (°P)</td>
<td>168.5</td>
<td>178.6</td>
<td>43.6</td>
<td>1.21 b</td>
<td>0.90 d</td>
<td>1.10 c</td>
<td>1.34 a</td>
<td>1.22 a</td>
<td>0.95 b</td>
<td>1.24 a</td>
</tr>
<tr>
<td>Ethanol (% v/v)</td>
<td>63.6</td>
<td>39.1</td>
<td>7.5</td>
<td>5.19 a</td>
<td>5.10 b</td>
<td>4.94 c</td>
<td>4.86 d</td>
<td>5.04 b</td>
<td>5.11 a</td>
<td>4.91 c</td>
</tr>
<tr>
<td>Apparent Degree of Fermentation (%)</td>
<td>147.9</td>
<td>162.2</td>
<td>36.3</td>
<td>89.11 b (2.1)</td>
<td>91.51 a (1.3)</td>
<td>89.56 b (2.3)</td>
<td>87.35 c (1.0)</td>
<td>88.72 b (2.1)</td>
<td>91.15 b (2.0)</td>
<td>88.27 c (1.6)</td>
</tr>
<tr>
<td>Real Degree of Fermentation (%)</td>
<td>148.2</td>
<td>161.0</td>
<td>36.0</td>
<td>73.16 b (1.7)</td>
<td>75.00 a (1.0)</td>
<td>73.43 b (1.8)</td>
<td>71.70 c (0.8)</td>
<td>72.82 b (1.6)</td>
<td>74.71 a (1.6)</td>
<td>72.43 c (1.3)</td>
</tr>
<tr>
<td>Concentration of Original Extract</td>
<td>66.8</td>
<td>14.9</td>
<td>8.8</td>
<td>11.05 a (0.3)</td>
<td>10.61 b (0.1)</td>
<td>10.50 b (0.1)</td>
<td>10.59 a (0.1)</td>
<td>10.79 a (0.2)</td>
<td>10.68 b (0.2)</td>
<td>10.59 b (0.2)</td>
</tr>
<tr>
<td>Real Extract (°P)</td>
<td>166.8</td>
<td>146.6</td>
<td>43.4</td>
<td>3.10 a (0.2)</td>
<td>2.77 c (0.1)</td>
<td>2.91 b (0.1)</td>
<td>3.12 a (0.1)</td>
<td>3.06 a (0.2)</td>
<td>2.82 b (0.2)</td>
<td>3.04 a (0.1)</td>
</tr>
<tr>
<td>Ethanol (% w/w)</td>
<td>64.9</td>
<td>41.4</td>
<td>8.0</td>
<td>4.08 a (0.1)</td>
<td>4.02 b (0.1)</td>
<td>3.89 c (0.1)</td>
<td>3.83 d (0.0)</td>
<td>3.96 b (0.1)</td>
<td>4.03 a (0.1)</td>
<td>3.87 c (0.1)</td>
</tr>
<tr>
<td>Color (srm)</td>
<td>10.0</td>
<td>21.2</td>
<td>†</td>
<td>2.13 b (0.3)</td>
<td>2.51 a (0.2)</td>
<td>2.11 b (0.4)</td>
<td>2.45 a (0.3)</td>
<td>2.60 a (0.3)</td>
<td>2.22 b (0.3)</td>
<td>2.09 b (0.2)</td>
</tr>
<tr>
<td>pH</td>
<td>26.0</td>
<td>31.4</td>
<td>5.4</td>
<td>4.44 a (0.1)</td>
<td>4.39 a (0.1)</td>
<td>4.43 a (0.1)</td>
<td>4.30 b (0.1)</td>
<td>4.42 a (0.1)</td>
<td>4.42 a (0.1)</td>
<td>4.32 b (0.1)</td>
</tr>
<tr>
<td>International Bitterness Units (ppm)</td>
<td>79.6</td>
<td>25.4</td>
<td>9.5</td>
<td>9.74 b (2.2)</td>
<td>13.34 a (0.9)</td>
<td>14.03 a (0.9)</td>
<td>13.22 a (1.0)</td>
<td>13.67 a (1.3)</td>
<td>12.05 b (1.6)</td>
<td>12.03 b (2.8)</td>
</tr>
</tbody>
</table>

*** probability <0.001; NS (Not Significant) probability ≥ 0.05

† sums of squares for insignificant interaction added to error sums of squares

abcd mean values with the same letter within the same row within the same variable (variety, location) are not significantly different when a probability level of 0.05 is applied
Nuclear Magnetic Resonance (NMR)
### NMR Results

45 compounds and 38 Volatile Compounds
LC-QTOF-MS
Hierarchical Clustering Tree
n=180 compounds; ESI Pos

Adenine

Beer
Wort
Partial Least Square Discrimination Analysis (PLS-DA)

Correlations on axes t1 and t2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
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<tr>
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**CDC Bow**
- Propionic acid, ethyl ester (ppacee)
- Sweet, fruity, grape, pineapple

**Isobutyl acetate** (Ibace)
- Fruity

**Harrington**
- Hexanol (Hexol)
- Fruity Alcohol
Conclusions

• The developed model in our pilot study (4 varieties of beers and 3 location):

1. Showed a promising approach to study and to correlate the sensory data to other physical/chemical measurements
2. Might be useful to both barley and beer industry in developing new generation of beers with different varieties of barley
3. May be a useful tool to predict the sensory quality and consumer acceptability of final products
Acknowledgments

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• The Brewing & Malting Barley Research Institute
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- **The Canadian Grain Commission:** Véronique Barthet and Tao Fan (GC-MS)
- **My Team:**
  Donna Ryland
  April McElrea
  Shiva Shariati
  Erin Goldberg
  Ronak Fahmi
  Le Wang
Cheers
Michel Aliani
Michel.Aliani@Umanitoba.ca
Partial Least Square Discrimination Analysis (PLS-DA)

Correlations on axes t1 and t2

- **Isopentyl acetate**: (Fruity Aroma)
- **Alpha Calacorene**: (Woody)
- **Ethyl decanoate**: (Sweet, Apple)
- **Specific Gravity**
- **IBU**
- **Apparent extract**
- **Hop Flavour**

### Abbreviations for Volatiles
- 1-Butanol-3-Methyl acetate (**ButMA**)
- Ethyl decanoate (**EtDec**)
- Alpha Calacorene (**Acal**)
- Hexanoic acide (**HexAc**)
- Benzene-3 methylbutyl (**BenMB**)
- Octanoic acid (**OctAc**)
- Carvacrol [(Phenol, 2-methyl-5-(1-ethylmethyl)] - Car
- Butylated hydroxytoluene (**ButHT**)
- Murrorul (**Mur**)
- Cadinol (**Cad**)

### Volatiles
- **Isopentyl acetate**: (Fruity Aroma)
- **Alpha Calacorene**: (Woody)
- **Ethyl decanoate**: (Sweet, Apple)
- **Specific Gravity**
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