

**ASBC Annual Meeting**

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# **Comparison of the flavor and aroma compounds present in aging lambic beer**

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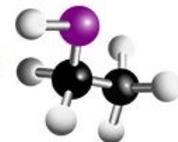
Southern Illinois University

# What is a lambic beer?

- Spontaneously fermented from 30% raw wheat
- Produced only in Pajottenland, Southwest of Brussels, Senne valley, and in Brussels
- Fermented for minimum of a year up to three years



Picture: [http://www.rooms-europe.de/english/belgium/map\\_belgium.html](http://www.rooms-europe.de/english/belgium/map_belgium.html)



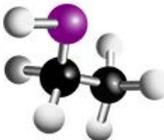
# Sensory profile

- Range in color from golden yellow to light amber
- High volatile acidity produces vinegar and goaty/cheesy aromas
  - Acetic Acid – LB 700 - 1500 mg/L vs AB 50 – 150 (mg/L)
  - Lactic Acid – LB 1500 – 3000 mg/L vs AB 40 – 150 (mg/L)
- Brettanomyces – common in red wine spoilage
  - Horsey, musky, or barnyard
- Taste – sour, acidic, not bitter, sweetness varies
- Commonly compared to a wine or cider



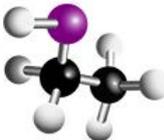
# Fermentation

- 4 Stages
- Stage 1: *Kloeckera. apiculata* and enteric bacteria
  - 3 – 7 days after the wort inoculated
  - Glucose
  - pH drop 5.1 to 4.6 increase acetic and lactic bacteria
- Stage 2: *Saccharomyces*
- Stage 3: Lactic and acetic acid
  - 3 – 4 an initial inoculation
  - Overlaps Stage 2
  - Warmer temperatures
- Stage 4: *Brettanomyces*
  - 8 months
  - Esterase converts acid and alcohol into esters ( Ethyl acetate and ethyl lactate)



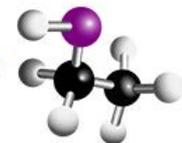
# Gas Chromatography – Olfactometry (GC-O)

- Analytical technique utilizes human nose as detector
- 10 – 30 compounds make up aroma profile
- Sulfur compounds low sensory threshold not typically detected by GC (ng/L)
- Aroma extraction dilution analysis
  - Extraction diluted (1:2 or 1:3)
  - Continue sniffing extracts until nothing detected
  - Requires no special software or computer
- OSME
  - Single sample
  - Require intensity transponder and software



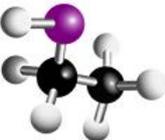
# Research objective

- Determine the major aroma compounds of aging lambic beer using GC-O



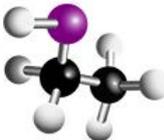
# Aging samples

- Samples
  - 3, 6, 9, 12 and 28 months
- Obtained from Lindemans brewery
  - Vlezenbeek, Belgium
- GC-MS
- GC-O



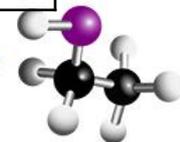
# Identification

- 41 compounds identified
- 30 compounds not previously reported in lambics
  - Table of compounds next slide
- 11 previously reported
  - Fatty Acids: hexanoic, octanoic, and decanoic acids
  - Esters: acetate, lactate, caprate, octanoate, decanoate
  - Phenethyl acetate, Phenethyl alcohol
  - Ethanol
- Brettanomyces
  - 4 ethylphenol, 4-ethylguaiacol



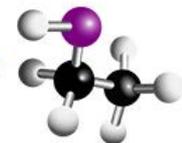
# Previously unreported compounds in lambics

Isopentyl alcohol	2-heptanone	Ethyl hexanol	4-ethylphenol	4-ethylguaiacol
2-methylbutanol	Heptan-2-ol	Isoamyl acetate	Nonanol	Ethyl nanonate
Ethyl isovalerate	Amyl acetate	Ethyl heptanoate	L-alpha-terpineol	Isoamyl octanoate
Isovaleric acid	2-Furanone	2-nonanol	Decanal	2-ethyl-hexanoic acid
Isoamyl alcohol	Heptanol	Nonanal	Decanol	Ethyl undecanoate
2-methyl-1-butyl acetate	Ethyl isohexanoate	2-ethyl-hexanoic acid	Ethyl benzoate	2-methylbutyl octanoate



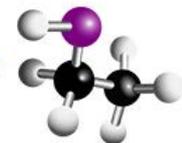
# GC-O

- 3 panelists
- Trained panelist using known aroma compounds
  - Develop a common lexicon
- Training lasted two weeks
- Ranked (very weak, weak, medium, strong, very strong)
- Standards and LRI values used to identify compounds
- 28 aroma compounds identified overall
- 3 month
  - 8 compounds
- 28 month
  - 17 compounds



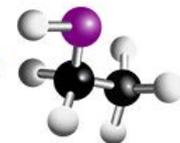
# 3 Month Sample (GC-O)

LRI	Compound	Descriptor	Ranking
658	Propionic acid	rancid	weak
745	Isoamyl alcohol	malt	weak
790	Ethyl butyrate	apple	very weak
875	Isovaleric acid	rancid	medium
880	Hexanol	green	medium
946	Ethyl isohexanoate	fruit	weak/medium
1019	Hexanoic acid	sweaty	weak
1065	Octanol	chemical	weak



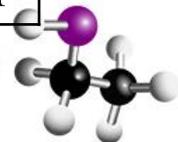
# 6 Month GC-O

<b>LRI</b>	<b>Compound</b>	<b>Descriptor</b>	<b>Ranking</b>
658	Propionic acid	rancid	weak
727	Amyl alcohol	malt	strong
790	Ethyl butyrate	bread	medium
875	Isovaleric acid	rancid	weak
902	Heptanal	fruit	medium
1019	Hexanoic acid	chemical	medium
1065	Octanol	fruit	very weak
1098	Ethyl heptanoate	musky	weak
1166	Ethyl benzoate	fruity	very weak



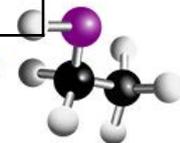
# 9 Month Sample GC-O

LRI	Compound	Descriptor	Ranking
593	Diacetyl	buttery	weak
587	Ethyl acetate		very weak
745	Isoamyl alcohol	malt	weak
833	Furfural (2-furanal)	bread	weak
875	Isovaleric acid	rancid	medium
880	Hexanol	green	weak
1010	Ethyl lactate	fruit	weak
1019	Hexanoic acid	sweaty	weak
1065	Octanol	chemical	weak
1168	Ethyl phenol	musky	medium
1204	Decanal	orange	medium



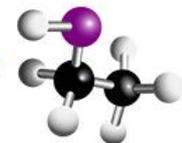
# 12 Month GC-O

LRI	Compound	Descriptor	Ranking
506	Dimethyl sulfide	sulfur, rotten	medium
593	Diacetyl	buttery	weak
658	Propanoic acid	rancid	weak
727	Amyl alcohol	burnt	medium
790	Ethyl butyrate	apple	weak
875	Isovaleric acid	rancid	weak
880	Hexanol	green	very weak
1019	Hexanoic acid	sweaty	medium
1098	Ethyl heptanoate	fruit	medium
1168	Ethyl phenol	musky	strong
1204	Decanal	Orange	weak



# 28 Month GC-O

LRI	Compound	Descriptor	Ranking
506	Dimethyl sulfide	sulfur, rotten	Medium
593	Diacetyl	buttery	Weak
727	Amyl alcohol	Burnt	very strong
745	Isoamyl alcohol	Malt	Strong
790	Ethyl butyrate	Apple	very weak
833	Furfural (2-furanal)	Bread	medium
875	Isovaleric acid	Rancid	strong
880	Hexanol	Green	weak
902	Heptanal	Rancid	weak
946	Ethyl isohexanoate	Fruit	very weak
1010	Ethyl lactate	Fruit	weak
1019	Hexanoic acid	Sweaty	very strong
1065	Octanol	Chemical	medium
1098	Ethyl heptanoate	Fruit	very weak
1168	Ethyl phenol	Musky	weak
1241	Benzeneacetic acid, ethyl ester	Fruit	very weak
1253	$\beta$ -Phenethyl acetate (Acetic acid, 2-phenylethyl ester)	rose, sweet	very weak

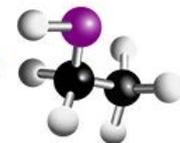


# Aroma compound ID in aging Lambic using GC-O

LRI	Compound	Age (months)					Descriptor
		3	6	9	12	28	
506	Diemethyl sulfide	--	--	--	M	M	sulfur, rotten
593	Diacetyl	--	--	W	W	W	buttery
587	Ethyl acetate	--	--	VW	--	--	fruity
658	Propanoic acid	W	W	--	W	--	rancid
727	Amyl alcohol	--	S	--	M	VS	burnt
745	Isoamyl alcohol	W	--	VW	--	S	malt
790	Ethyl butyrate	VW	M	--	W	VW	apple
833	Furfural (2-fural)	--	--	W	--	M	bread
875	Isovaleric acid	M	W	M	W	S	rancid
880	Hexanol	M	--	W	VW	W	green
902	Heptanal	--	M	--	--	W	rancid
946	Ethyl isohexanoate	W/M	--	--	--	VW	fruit
1010	Ethyl lactate	--	--	W	--	W	fruit
1019	Hexanoic acid	W	M	W	M	VS	sweaty
1065	Octanol	W	VW	W	--	M	chemical
1098	ethyl heptanoate	--	W	--	M	VW	fruit
1166	ethyl benzoate	--	VW	--	--	--	fruity
1168	ethyl phenol	--	--	M	S	W	musky
1204	Decanal	--	--	M	W	--	orange peel
1241	Benzeneacetic acid, ethyl ester	--	--	--	--	VW	fruit
1253	$\beta$ -Phenethyl acetate (Acetic acid, 2-phenylethyl ester)	--	--	--	--	VW	rose, sweet

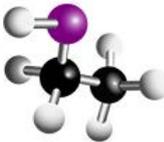
VW: very weak, W: weak, M: medium, S: strong, VS: very strong

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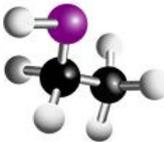
# Compounds

- Fusel alcohols
  - Isoamyl, amyl, hexanol
- Oxidative hops
  - Isovaleric acid
- Brettanomyces
  - Development of 4-ethylphenol after primary and secondary fermentation has been completed
  - Ethyl isovalerate not detected by panelist but was by GC-MS



# Concentrations

- 4-ethylphenol (threshold 0.3 ppm in beer)
  - 9 months (4.0 ppm)
  - 12 months (8.82 ppm)
  - 28 months (1.0 ppm)
- 4-ethylguaiacol
  - Not detected by the panelist



# Literature Cited

- Guinard, J. X., *Lambic*. Brewers Publications: Boulder, 1990; p 159
- Nickerson, G. B.; Likens, S. T., Gas chromatographic evidence for the occurrence of hop oil components in beer. *Journal of Chromatography* **1966**, 2, 677-678.
- Engel, W.; Bahr, W.; Schieberle, P., Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, 209, 237-241.
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