Quantification of odour active compounds and calculation of their orthonasal and retronasal detection thresholds in alcohol-free beer

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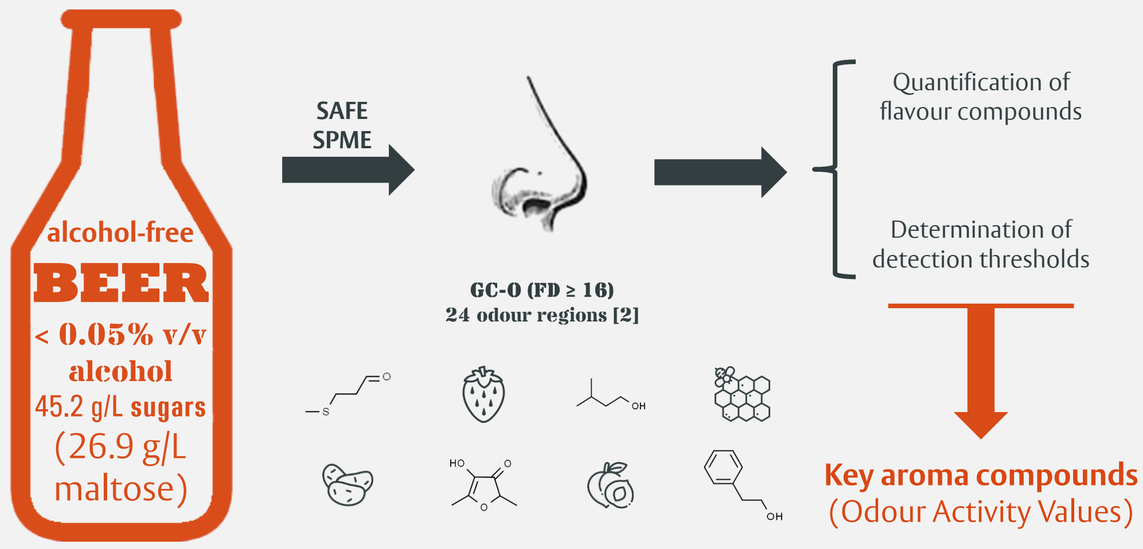
Introduction

- Alcohol-free beers brewed by cold contact fermentation exhibit a **flavour reminiscent of wort** [1].
- Their **low alcohol** content (<0.05% v/v) and the high content of **sugars** alter the release properties of these beers, and thus the perception threshold of flavour compounds.
- Different **threshold calculation methods** were found in the literature, but the effect of the different methods has not been studied yet.

Aims of the study

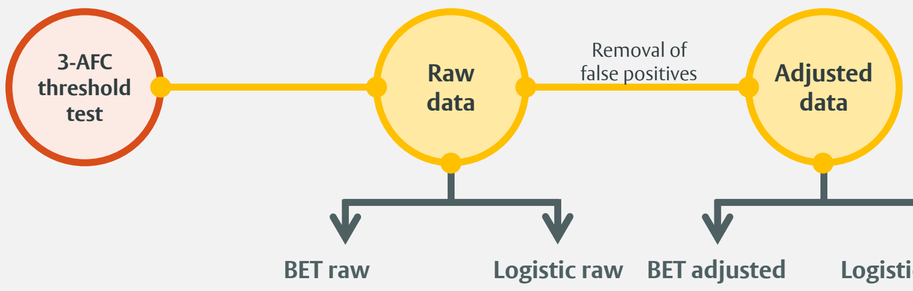
- to determine **orthonasal and retronasal detection thresholds** of flavour compounds in a model alcohol-free beer.
- to check the effect of the **threshold calculation method.**
- to find their **odour activity values** (OAV) in a reference alcohol-free beer.



Materials & Methods

Detection thresholds

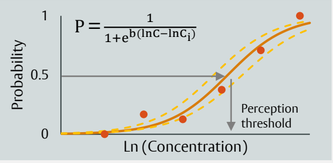
Thresholds were calculated by four different methodologies:
either Best Estimate Threshold (BET) or logistic regression, from raw data or with the false positives removed ('adjusted data')



$$\text{Best Estimate Threshold} = \sqrt{C_n \cdot C_{n+1}}$$

BET is calculated as the geometrical mean of the concentrations for the higher negative response (C_n) and the next positive one (C_{n+1})

Logistic regression: Thresholds were calculated as the concentration at which 50% of the panellists gave a correct response.



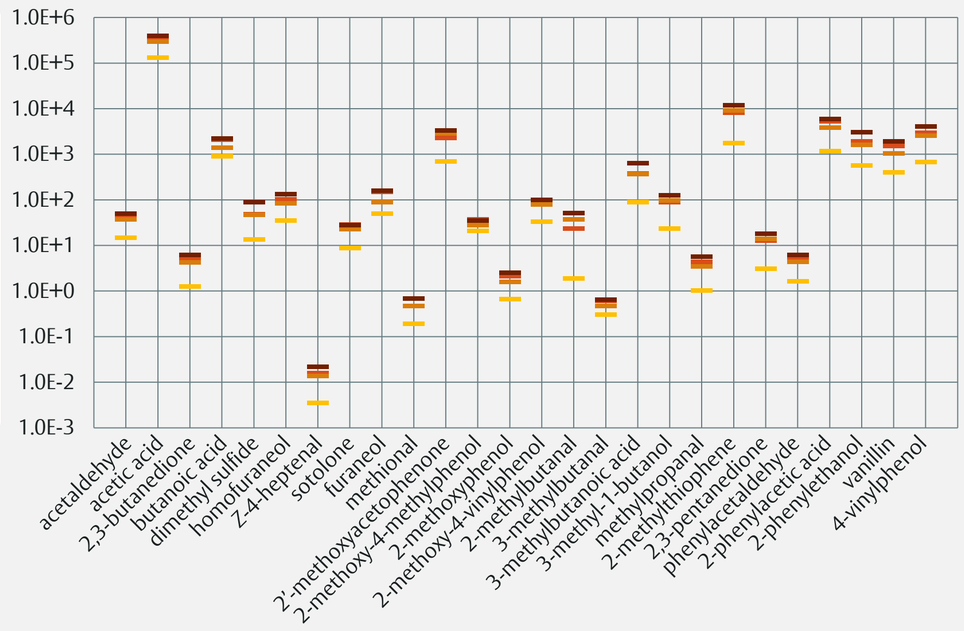
Quantification of flavour compounds

HS-SPME-GC-MS	**Acetic acid, butanoic acid, 3-methylbutanoic acid, 3-methyl-1-butanol, 2-phenylethanol, 2-phenylacetic acid, dimethyl sulfide, 2,3-butanedione, 2,3-pentanedione, acetaldehyde**. SPME fibre: DVB/CAR/PDMS. GC column: Stabilwax®-DA.
PFBHA-HS-SPME-GC-MS	**Methional, methylpropanal, 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, *Z*-4-heptenal**. The SPME fibre was exposed to O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) prior to be exposed to the sample's headspace. Fibre: PDMS/DVB. GC column: VF17MS.
HPLC-UV	**4-Vinylguaiacol.** UV detection at 260 nm. LC column: Supelcosil Abz+ (250x4.6 mm).
UPLC-FLR	**4-Vinylphenol**. Fluorescence detection: excitation at 257 nm, emission at 334 nm. LC column: Acquity UPLC® BEH C18(1.7 µm, 2.1x150 mm).
LC-MS/MS	**Sotolone** (129.13→82.18), **furaneol** (129.1→43.1), **homofuraneol** (143.13→69.07), **vanillin** (153.10→93.10). Numbers in brackets correspond to MS/MS transitions. LC column: Acquity UPLC® BEH C18(1.7 µm, 2.1x150 mm).
Dansylation-LC-MS/MS	**2-Methoxyphenol** (358.1→171.1), **2-methoxy-4-methylphenol** (372.1→171.1). Hydroxyl groups were derivatised with dansyl chloride. MS/MS transitions of the derivatised compounds in brackets. LC column: Agilent Zorbax® SB-18(1.8 µm, 2.1x100 mm).

Results & Discussion

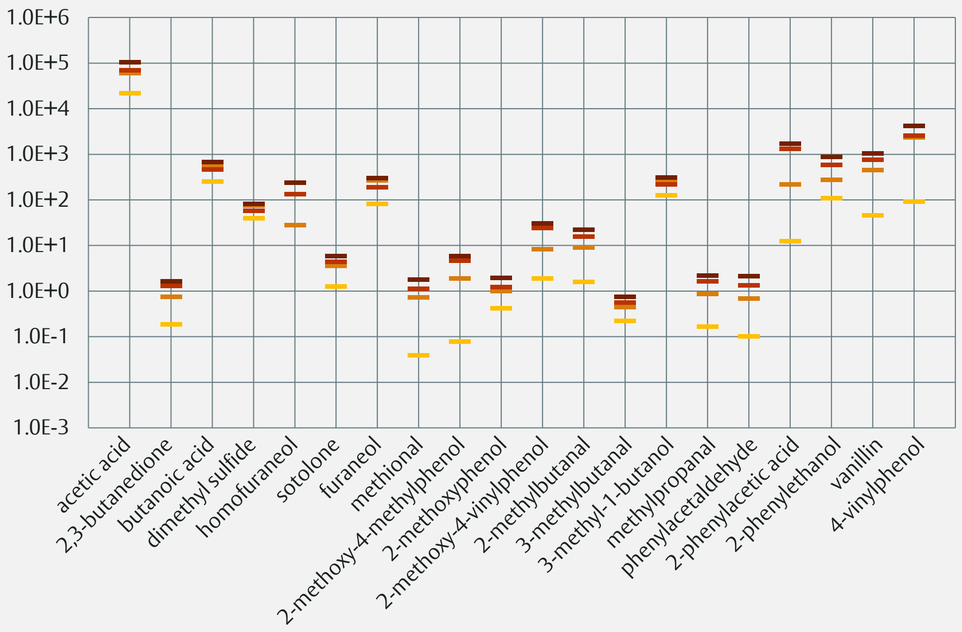
Orthonasal detection thresholds, µg/L

Four different calculation methods:
- BET adjusted
- BET raw
- Logistic adjusted
- Logistic raw

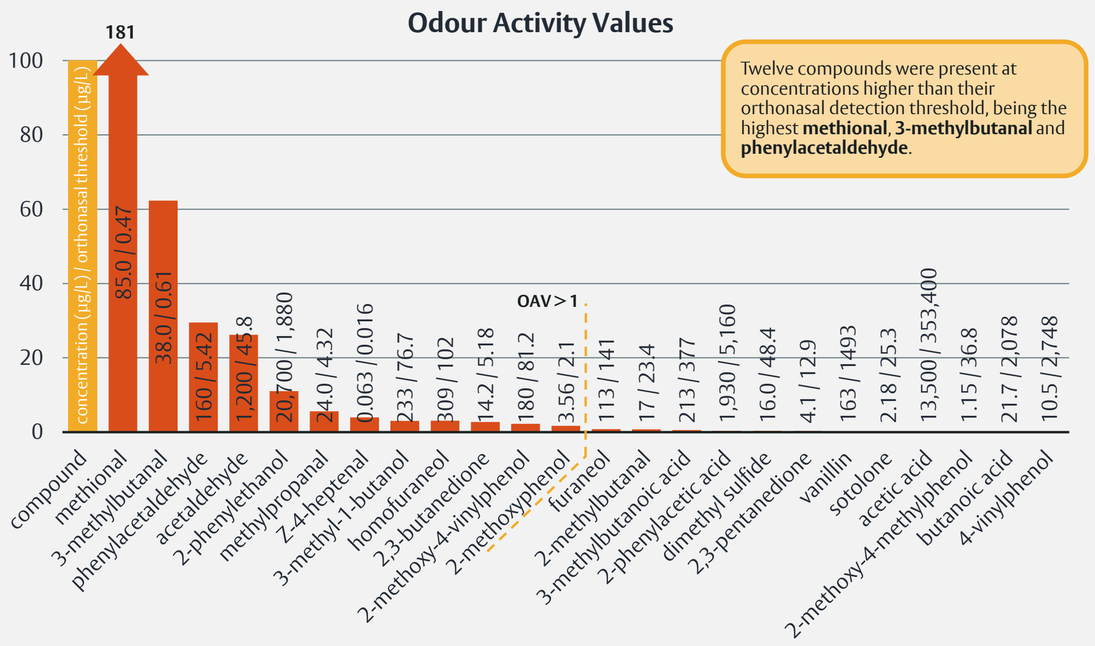


Retronasal detection thresholds, µg/L

- Both **orthonasal** and **retronasal** detection thresholds were distributed across a broad range of values.
- **Differences** between calculation methods were higher for retronasal thresholds due to the higher variability of retronasal outcomes.



Odour Activity Values



Twelve compounds were present at concentrations higher than their orthonasal detection threshold, being the highest **methional**, **3-methylbutanal** and **phenylacetaldehyde**.

The compounds with highest concentrations were **2-phenylethanol** (20,700 µg/L), **acetic acid** (13,500 µg/L), **2-phenylacetic acid** (1,930 µg/L), and **acetaldehyde** (1,200 µg/L). On the other hand, the ones with the lowest concentrations were ***Z*-4-heptenal** (0.063 µg/L), **2-methoxy-4-methyphenol** (1.15 µg/L), and **2,3-pentanedione** (4.1 µg/L).

Conclusions

- Threshold values were dependent on the calculation method chosen, as well as on the treatment of the data for the removal of false positives.
- **Strecker aldehydes** were found to be of great importance for the worty, malty aroma of alcohol-free beers brewed by cold contact fermentation.
- The results from our study will help brewers and beer science researchers understand the contribution of aroma compounds to alcohol-free beer, and thus improve quality and consumer acceptance.

Want to know more details of our threshold study?
Check out our last publication!

https://qrs.ly/ur9xq9f

References.1. Perpète & Collin, *Food Chem*, 71(3), 379-385, **2000**. **2.** Piornos et al., *Flavour Science. Proceedings of the XV Weurman Flavour Research Symposium*, Siegmund & Leitner, Eds. Verlag der Universität Graz, 343-346, **2017**.

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