2014 ABSC Annual Meeting in Chicago

Tracking oxidative degradation of linoleic acid by incorporation of isotope labels in aroma active products *cis*- and *trans*-4,5-Epoxy-2*E*-decenal

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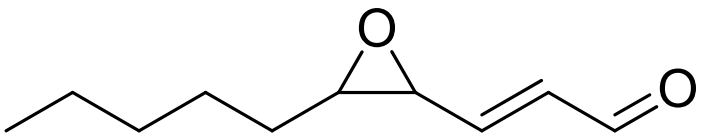
The Science of Beer

Introduction/Background

- Complex matrix of beer is subject to degradation processes during aging
- Oxidative degradation processes of e.g. fatty acids
- Leading to staling and off flavor

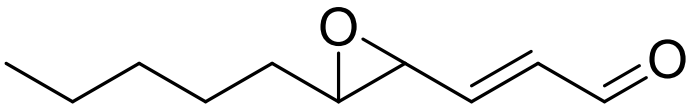
Representative species of off-flavor contributing compounds

very low odor thresholds of 15 ng/L water (0.015 ppb) for the *trans*-4,5-Epoxy-2*E*-decenal



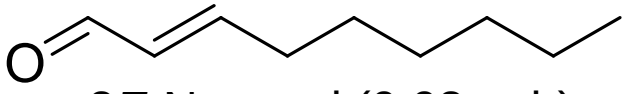
cis-4,5-Epoxy-2*E*-decenal

malty - sweet - fruity



trans-4,5-Epoxy-2*E*-decenal

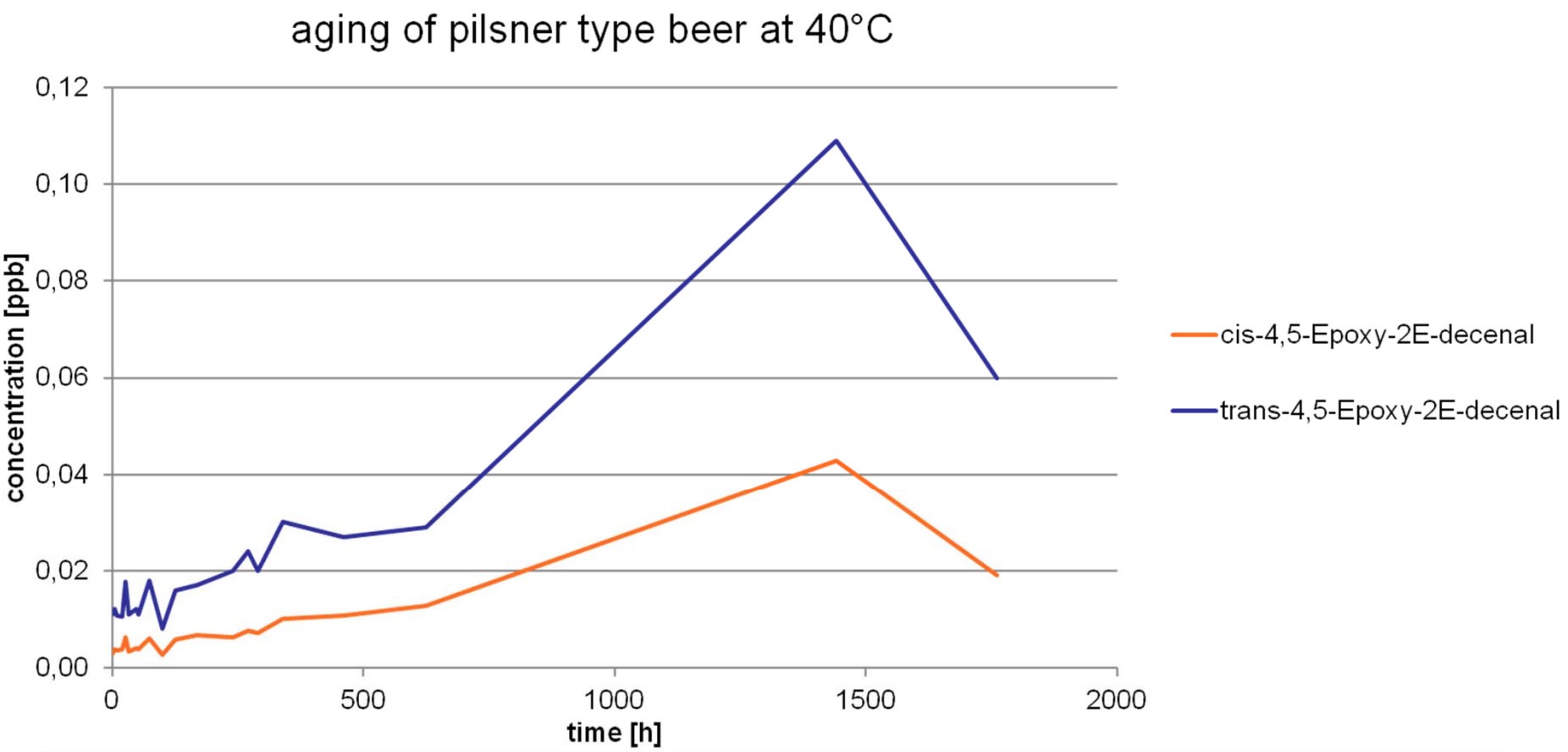
metallic - fruity



2*E*-Nonenal (0.08 ppb)

cardboard

Formation of Epoxydecenal during forced aging



How to explain the appearance of Epoxydecenals?

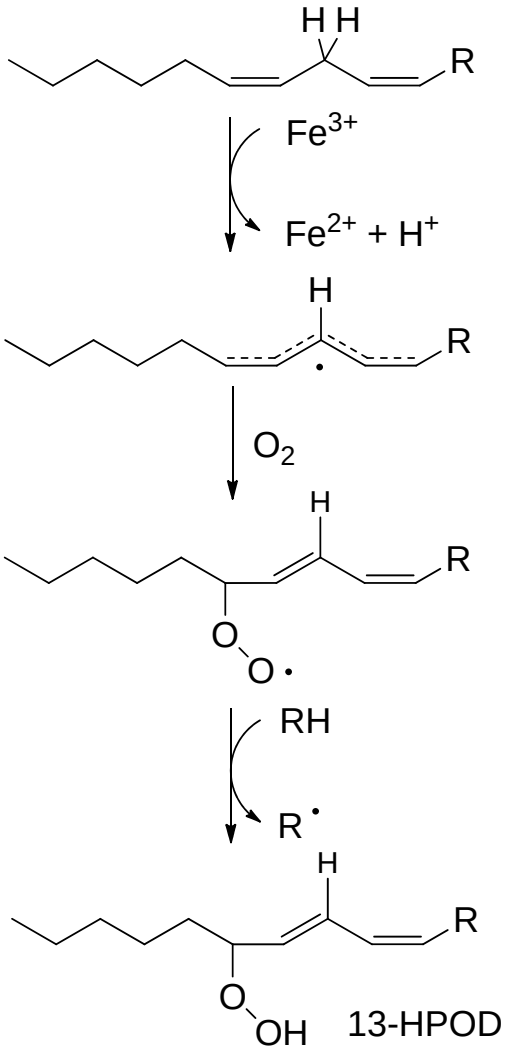
two models:

- *de novo* formation
- release from ´masked´ Epoxydecenals formed during malting or mashing

De novo formation in beer

- Beer contains linoleic acid roughly at 0.1 ppm levels
- Autoxidation processes occur, triggered e.g. by Fe-ions
- Radical chain reaction leads to aroma active products like Epoxydecenals

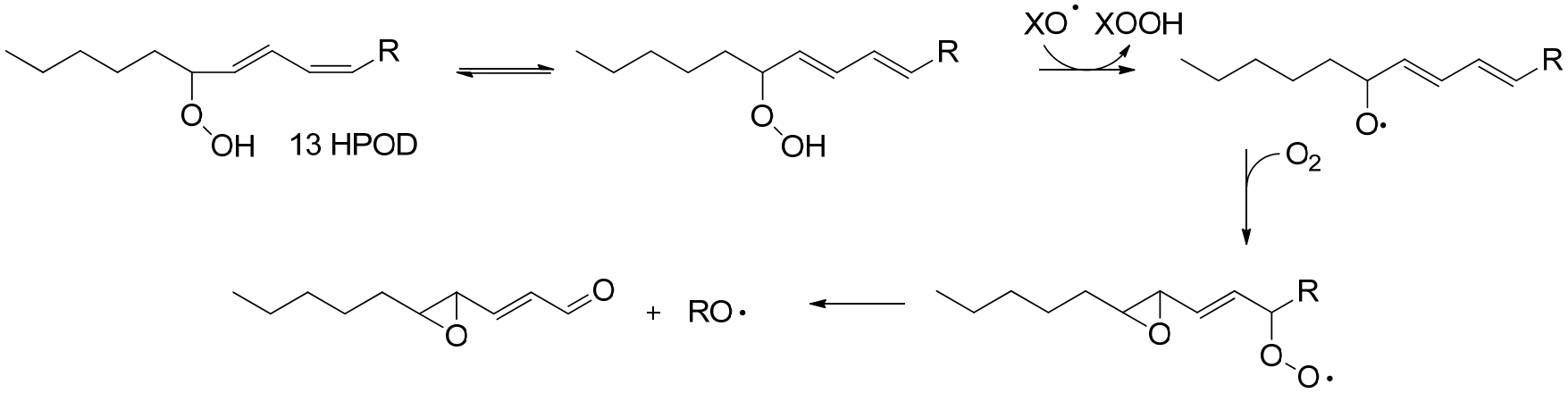
De novo formation in beer



First steps of autoxidation:

- Oxidation of fatty acid to acylradical
- Incorporation of one O_2 molecule
- Reaction with a 2nd fatty acid molecule

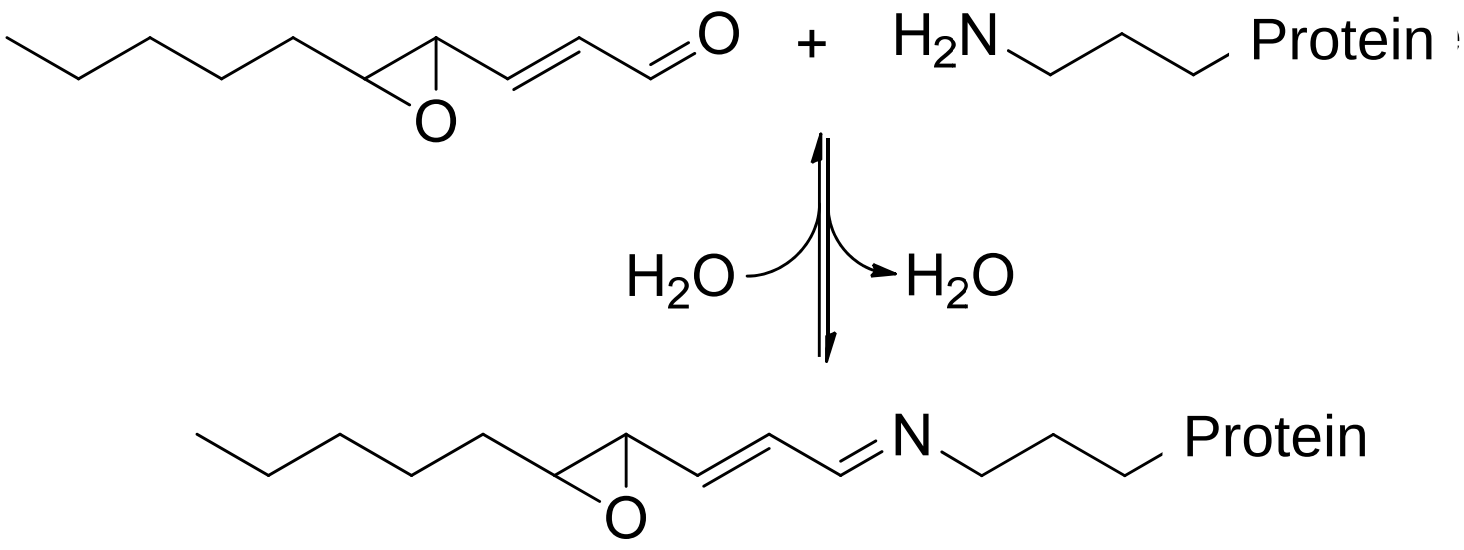
De novo formation in beer



The proposed mechanism of the degradation of 13-HPOD leading to Epoxydecenal by incorporation of a 2nd oxygen molecule.

Release from ´masked´ Epoxydecenals

How to mask and de-mask Epoxydecenals originating from LOX activity during malting and mashing?



Schiff base

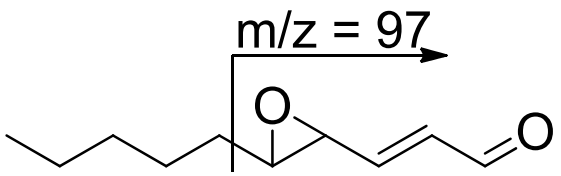
Is it possible that linoleic acid is degraded to Epoxydecenal in beer during aging?

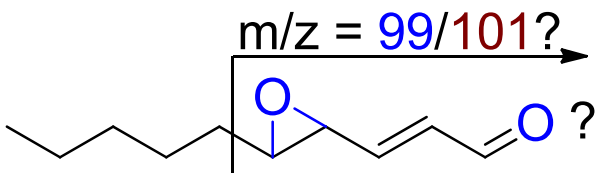
1. adding uniformly ^{13}C-labelled linoleic acid under synthetic air and nitrogen atmosphere
2. flushing bottle headspace with $^{18}O_2$, add unmarked linoleic acid to one set and 5% (v/v) ethanol to the other (blank, data not shown)

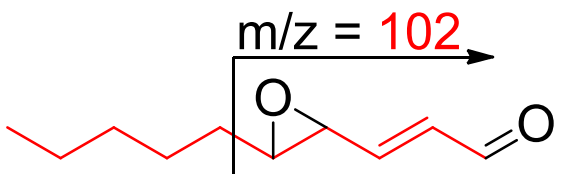
⟹ incubation at 40 °C, sampling at certain time, work-up (SPE), GC-NCI-MS (Methane)

Analysis

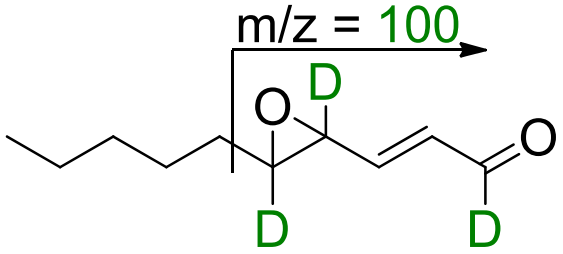
NCI-MS fragmentation pattern of the expected Epoxydecenals (only *trans*-form shown):







deuterated internal isotope standard
for semiquantitative analysis (1 ppb)

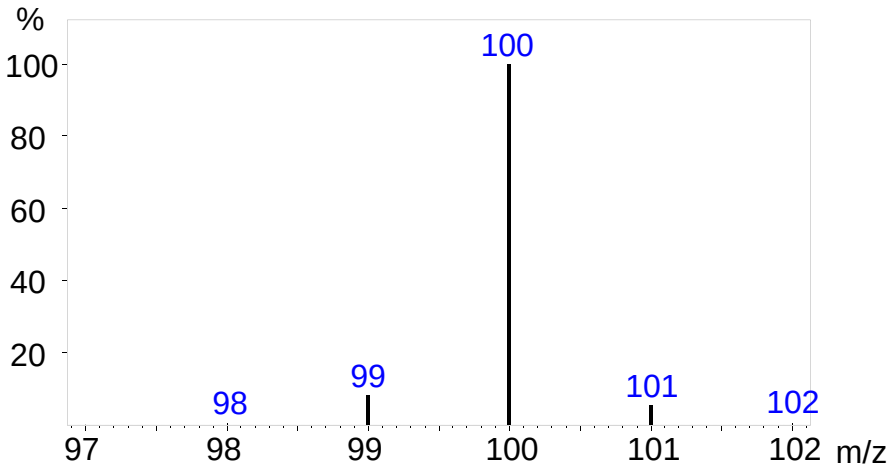


Very narrow scan range from 97 to 102 was used, so high sensitivity could be achieved.

Mass spectrum of triple deuterated epoxydecenal standard

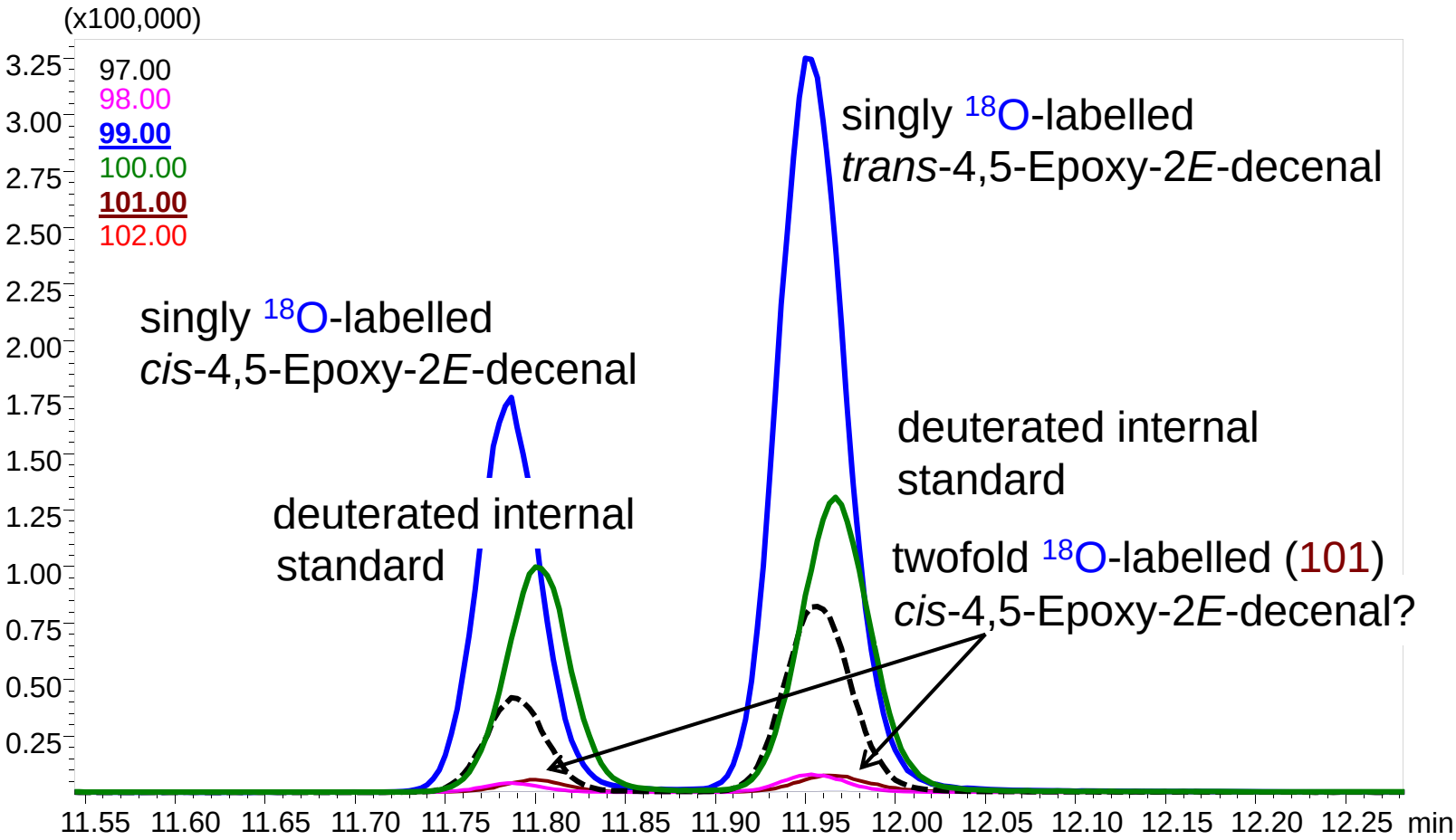
Low interference with mass fragments of targeted analytes:

- 99 and 101 for $^{18}O_2$-incubation
- 102 for ^{13}C-incubation



Masses 99 and 101 are present in the internal standard spectrum (ca. 5-6% of base peak).

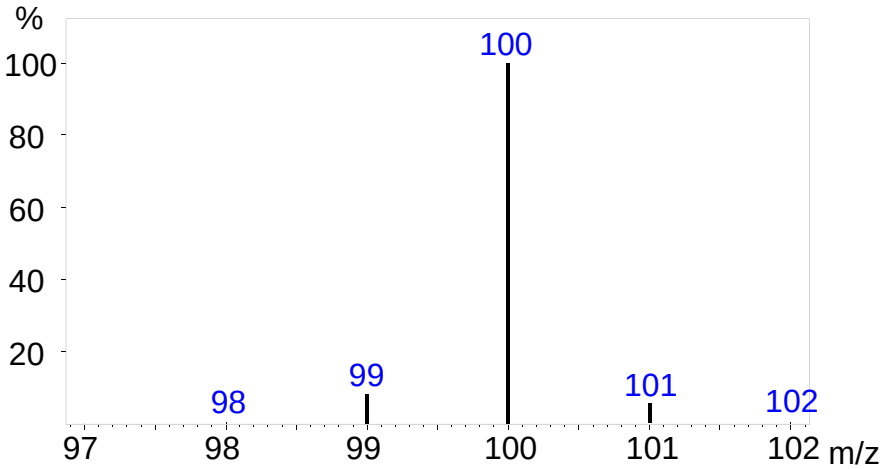
Mass traces of $^{18}O_2$-incubation 72h at 40°C



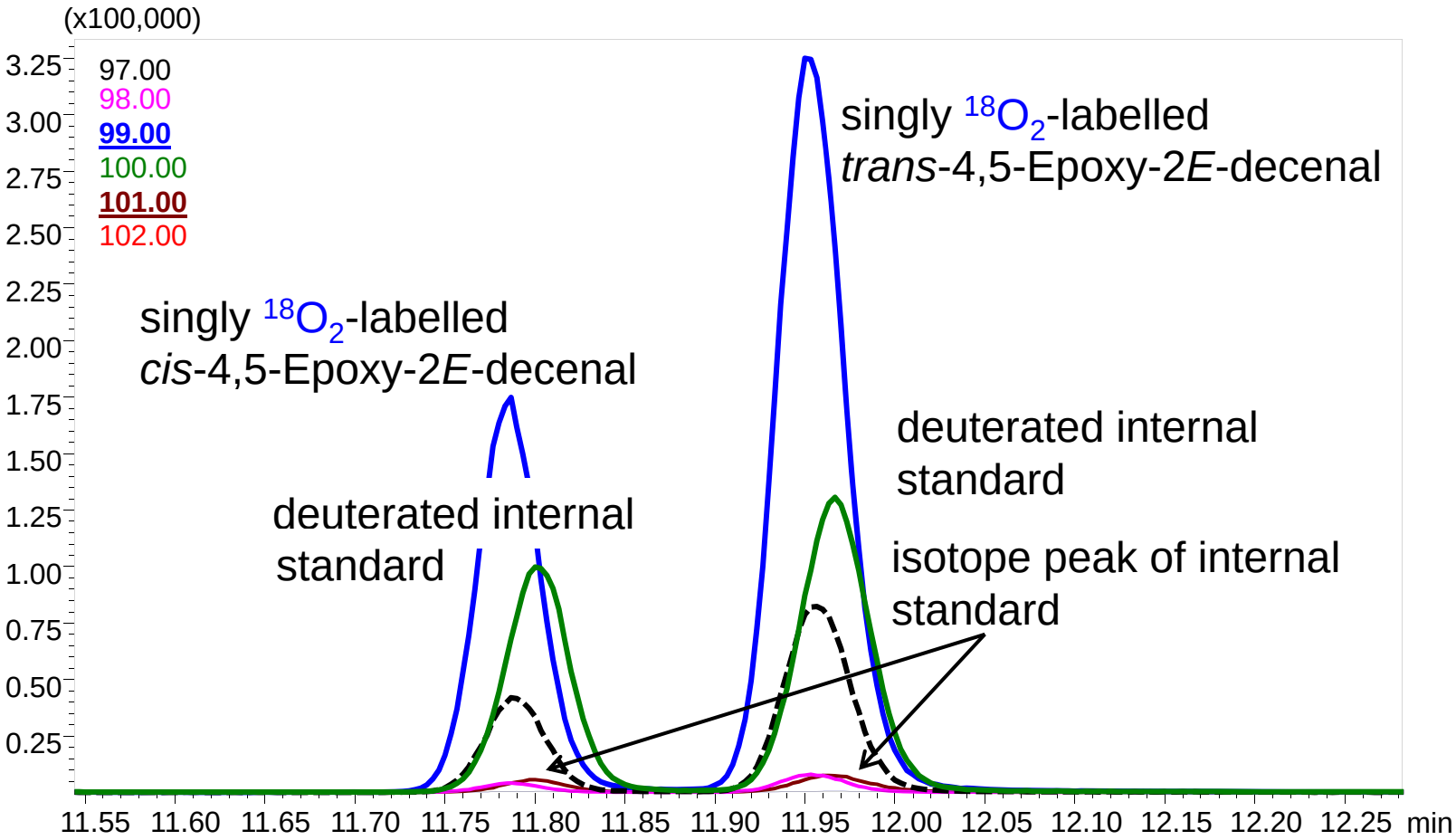
Assigning mass traces to the analytes

	rt	m/z	Area	%
3D-4*Z*-Epoxydecenal	11.801	100	257182	
3D-4*E*-Epoxydecenal	11.966	100	356966	
3D-4*Z*-Epoxydecenal +1	11.799	**101**	12900	5
3D-4*E*-Epoxydecenal +1	11.963	**101**	21559	6

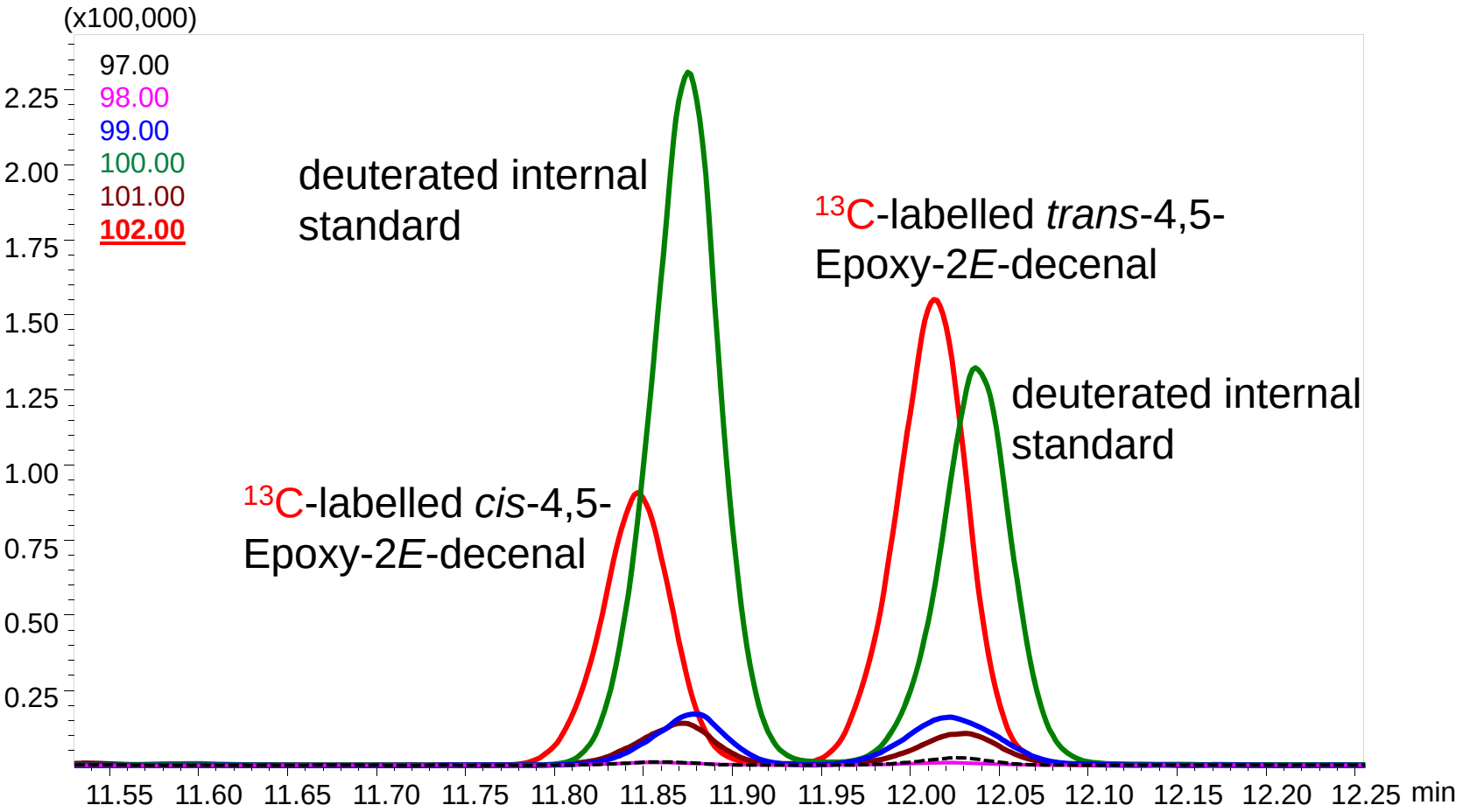
Peak areas for m/z 101 of around 5-6% of the peak area of m/z 100 belong to the internal standard and are NOT ascribable to the presence of two incorporated ^{18}O atoms.



Mass traces of $^{18}O_2$-incubation 72h at 40°C



Mass traces of ^{13}C-incubation under synthetic air, 24h at 40°C



First results

- ^{13}C-labels can be found in degradation products *trans*- and *cis*-Epoxydecenal after incubation of ^{13}C-labelled linoleic acid
- only one ^{18}O-label can be found in Epoxydecenals after incubation of linoleic acid with $^{18}O_2$

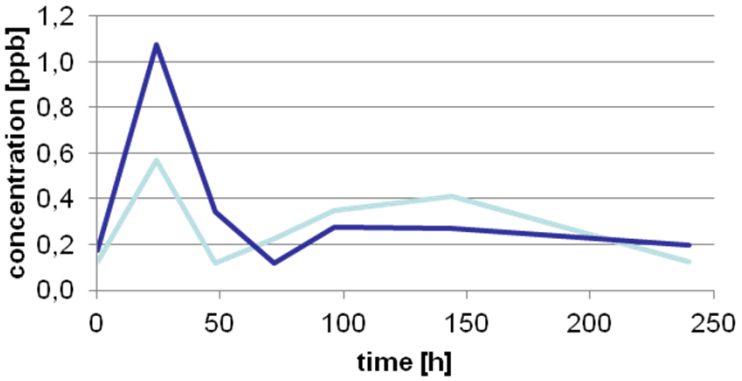
⇒ Linoleic acid is a precursor of Epoxydecenals

⇒ O_2 from bottle headspace or dissolved in beer is incorporated into Epoxydecenal

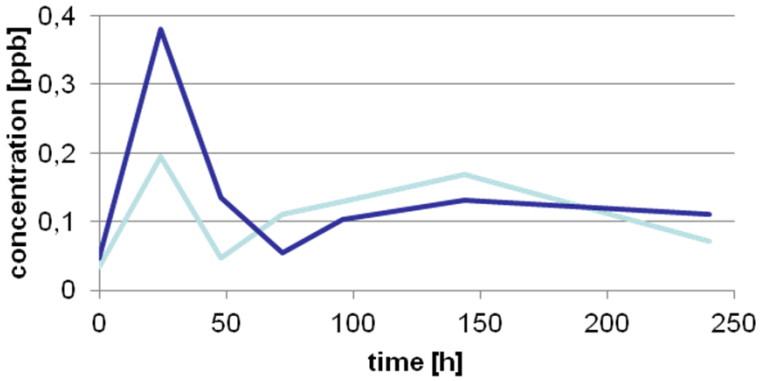
⇒ Exchange reactions on C1 occur with water in beer

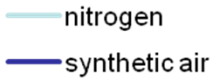
Incubation of ^{13}C-labelled linoleic acid

trans-^{13}C-Epoxydecenal

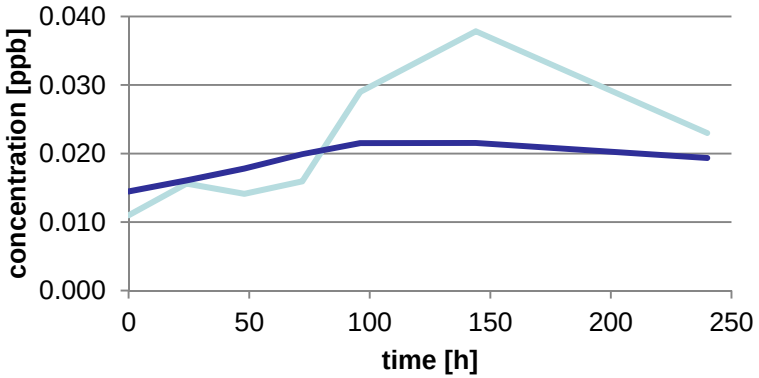


cis-^{13}C-Epoxydecenal

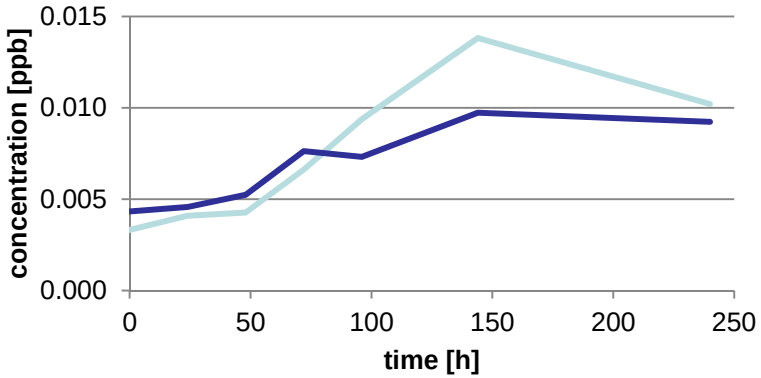


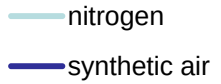


trans-Epoxydecenal

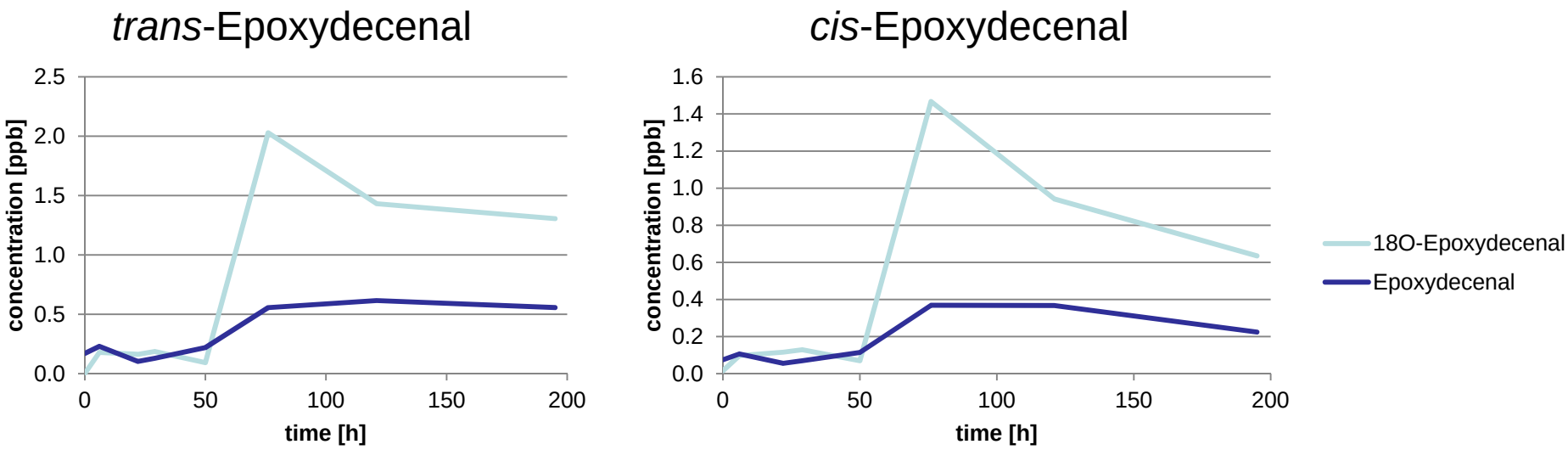


cis-Epoxydecenal





Incubation of linoleic acid under an $^{18}O_2$-atmosphere



Comparison of *cis*-Epoxydecenal concentrations

- Concentration peak of 1.1/0.6 ppb for incubation of ^{13}C-labelled linoleic acid under synthetic air/nitrogen
- Maximum concentration of 2 ppb for incubation of linoleic acid under ^{18}O-headspace

Comparison of *trans*-Epoxydecenal concentrations

- Concentration peak of 0.4/0.2 ppb for incubation of ^{13}C-labelled linoleic acid under synthetic air/nitrogen
- Maximum concentration of 1.4 ppb for incubation of linoleic acid under ^{18}O-headspace

Conclusion

- Formation of Epoxydecenal isomers under consumption of oxygen and linoleic acid in beer is possible.
- Oxygen seems to be a limiting factor.
- These stable isotope experiments are suitable to monitor the origin of aroma compounds.

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Thank you for your attention!

Exchange reaction

- Carbonyl function reacts with water to the 1,1-diol
- Water is released again
- Label remains 50%
- Excess water leads to decrease of ^{18}O-label