Lipid Oxidation

Materials:

- 2x Eppendorf Tube Racks
- Glasbox
- Quenching Buffer
- Methanol MeOH
- Isopropanol IPA
- Butylhydroxytoluol BHT

1. Quenching Buffers

1.1. MeOH:IPA 1:1 BHT (3 mM)

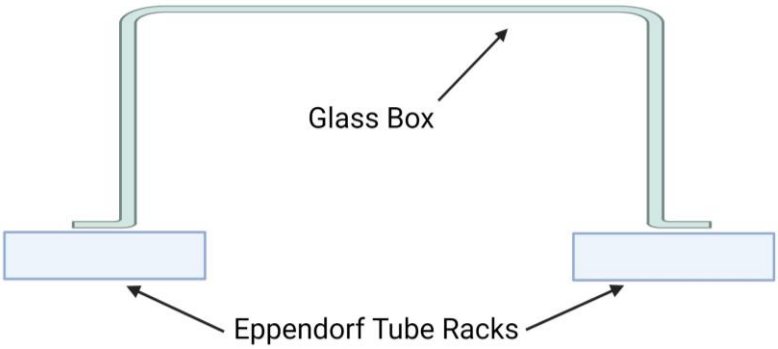
- MeOH:IPA 100 mL
- BHT: 66.7 mg

1.2. MeOH:IPA 1:1 BHT (30 uM)

- MeHO:IPA 99 mL
- MeOH:IPA + BHT (3 mM): 1 mL

2. Protocol

2.1. Construct for Air Oxidation



2.2. Quenching of the Samples

- Dried Lipid samples are air oxidized under the construct above
- Add 400 uL MeOH:IPA + BHT (30 uM) to each sample at end of the reaction
- Vortex Samples: 1min
- Incubation in Thermomix:
    - Max speed
    - 24°C
    - 10 min
- Freez Samples at -80°C

2.3. Experimental Setup

18 Samples are prepared in total (Eppendorf tubes with dried lipids). For each time point of sampling triplicates are used for statistical power. The Air oxidation is carried out to all 18 samples. At each of the following time points triplicates are quenched and frozen to evaluate the oxidation by LC-MS.

- Day 0
- Day 3
- Day 7
- Day 14
- Day 21
- Day 28