# Materials & Methods

1. Materials

Amine-functionalized polymers (AFP) and hydrophobic resins were purchased from Sigma Aldrich. The AFP were selected based on their functional group; aminomethyl, diethylenetriamine, sulfonyl amide, tris-(2-aminoethyl)amine, 2-mercaptoethylamine and ethylenediamine were tested. For the polymeric resins, the AmberliteTM XADTM series (4, 7HP, 16N), SepabeadsTM SP20SS and SP850 as well as DianonTM HP-2MG were investigated, differing in pore and particle size, surface area, and polymer type. CeoBeads with a molar C/N ratio of > 50 were purchased from HBU (HICeo  2000), the CeoBead (419095) from Sigma Aldrich and CB 374697 and CB926409 from CeoRise. All chemicals were purchased in analytical grade from Sigma Aldrich. Wort and alcohol-free beer produced by restricted fermentation were obtained from a local brewery. Original extract, alcohol-content and the concentration of iso-α-acids are specified in Table 1.

Table 1: Specification of original extract, alcohol and iso-α-acids content in wort and alcohol-free beer

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Wort** | **NAB** |
| Original extract [°P] | 16.68 | 12.00 |
| Alcohol [vol. %] | <0.06 | <0.06 |
| Total iso-α-acids [mg/L] | 36.8 | <0.04 |

1. Design of Experiments

The experimental strategy for the adsorbent selection persuaded in this work is outlined in Figure 1. In the first step, the adsorptive aldehyde removal of 16 resins was investigated in a complex, hopped wort base. Phase ratios were varied between 25 to 500 g/g and obtained fractions were additionally analysed for change in pH, colour and reusability. For selected batches, total fermentable sugars, iso-α-, and amino acids were measured.

4 resins

16 resins

2 resins

Figure 1: Sequential experimental design approach

In the second step, the four most promising adsorbents were selected and experiments based on a mixture-amount design (MinitabTM) were performed with a fixed phase ration of 100 g/g. An overview on the concentration ratios of all components at the low (-1), centre (0) and high (+1) level is given in Table 2. The selected amounts of total aldehydes are 475, 940, 1400, 1800, 4500 and 9000 μg/L, respectively. The chosen boundary conditions of the design are based on historic data of wort and non-alcoholic beer.

Table 2: Overview on mixture-amount design

|  |  |  |  |
| --- | --- | --- | --- |
| Component | Level -1 [%] | Level 0 [%] | Level +1 [%] |
| 2-methylpropanal | 5.0 | 5.8 | 6.6 |
| 2-methylbutanal | 1.7 | 4.2 | 6.7 |
| 3-methylbutanal | 8.0 | 19.1 | 30.2 |
| Hexanal | 0.1 | 0.2 | 0.2 |
| Methional | 9.3 | 10.6 | 11.9 |
| Phenyl acetaldehyde (aromatic) | 4.1 | 6.0 | 8.0 |
| Trans-2-nonenal | 0.02 | 0.02 | 0.02 |
| Benzaldehyde | 0.4 | 0.4 | 0.5 |
| Furfural | 36.0 | 53.7 | 71.3 |

In the last step, model robustness studies were performed by spiking possibly interfering molecules in batch uptake tests. The focus was to determine the impact of polyphenols (represented by catechin), diketones (represented by diacetyl), and other aldehydes (represented by acetaldehyde) on the adsorption efficiency.

1. Methods
   1. Batch uptake experiments

Prior to each batch uptake experiment, adsorbents were washed in four consecutive steps with 70 vol. % of ethanol and three volumes of Milli-Q water. CeoBead resins were dried additionally for 20 min at 180°C to remove any residual liquid. The required amount of wet or dry resin was then weighed into a 40 mL screw top vial (Supelco). Next, 35 g of wort or non-alcoholic beer were added to the vial, spiked were applicable, and the batch was stirred with the closed screw top overnight in a water bath at 15 °C to reach equilibrium. The liquid was then separated from the adsorbent by centrifugation and transferred into a fresh glass vial for analysis.

* 1. Resin regeneration

In order to assess the reusability of the tested adsorbents, resins were regenerated by incubating 1.4 g overnight at room temperature in a 35 mL of a 0.03647 wt. % HCl solution. After neutralisation with 2 M NaOH solution, the resins was rinsed with Milli-Q water, filtered and used for the batch uptake procedure described in section

* 1. Analysis of volatile aldehydes

Strecker aldehydes were analysed by headspace solid-phase micro-extraction (HS-SPME) based on a modified method of Vesely *et al*. [1] in a GC-MS (Agilent 7890A and 5975C MSD) and a 30 m x 0.25 mm x 0.25 μm VF17MS column. The derivatization reaction was carried out with O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBOA). Helium was used as the carrier gas at a flow rate of 1 mL/min. Characteristic mass fragment were identified for each molecule in order to ensure specific determination of the compound. The calibration was carried out for each medium in order to account for the matrix effects on the measurement.

* 1. Analysis of amino acids, fermentable sugars and iso-α-acids

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* 1. Other analysis methods

For a quantitative measurement of changes in the liquid the pH and the colour change of the liquid were measured. The colour change was measured in OD with a spectrophotometer at 420 and 450 nm. A leaching test for the amine-functionalized resins was performed using a LCK138 Hach test kit for total N-content (measuring range of 1-16 mg/L). The resins were washed as described and incubated in an HCl solution at pH 5.1. Hereafter, the resin was separated from the liquid, which were analysed for total N-content with a Hach DR3900 VIS spectrophotometer according to the method described by the manufacturer.

1. Vesely, P., et al., *Analysis of Aldehydes in Beer Using Solid-Phase Microextraction with On-Fiber Derivatization and Gas Chromatography/Mass Spectrometry.* Journal of Agricultural and Food Chemistry, 2003. **51**(24): p. 6941-6944.