



Chair of Bioseparation Engineering

Technical University of Munich

TUM

P22

Concentration and Extraction of Natural 4-Anisaldehyde, 3,4-Dimethoxybenzaldehyde and Benzaldehyde From Fermented Acid Whey

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Introduction & Motivation

Acid whey, a nutrient-rich by-product of the dairy industry, poses disposal challenges due to its sour taste and high volatile and lactic acid contents. In this project, acid whey was "fermented" with *Bacillus subtilis* to produce natural aroma compounds, such as benzaldehyde, 4-anisaldehyde, 3,4-dimethoxybenzaldehyde, benzylaldehyde and vanillin aldehyde (3,4-dimethoxybenzaldehyde). The aim of this project is to recover these compounds from the fermentation broth using reversed-phase (RP) preparative chromatography and finally, scale-up the process.

Chromatogram showing recovery of aromatic compounds from acid whey

Concentration of key aroma compounds — small scale

Initially, using a chromatography workstation, we compared two polymeric stationary phases (Oasis HLB and Phenomenex RP) for their performance in solid phase extraction, and determined their separation performance. Advantages of these stationary phases include:

- good chemical stability across a broad pH range
- compatibility with standard column procedures

For the extraction of aroma compounds, an ethanol-water mixture (90% ethanol, v/v) was used due to its toxicity, high extract efficiency, and suitability for food-grade applications.

Concentration of key aroma compounds — large scale

Oasis HLB performed better on smaller scale and was thus used for concentration of the key aroma compounds on larger scale (4 L of fermented acid whey).

Comparison of extraction methods

| Method | Retention time (min) | Recovery (%) |
|---------------|----------------------|--------------|
| Oasis HLB | ~10 | ~90 |
| Phenomenex RP | ~10 | ~60 |
| RP-18 | ~10 | ~60 |
| RP-18 | ~15 | ~60 |
| RP-18 | ~20 | ~60 |
| RP-18 | ~25 | ~60 |
| RP-18 | ~30 | ~60 |
| RP-18 | ~35 | ~60 |
| RP-18 | ~40 | ~60 |
| RP-18 | ~45 | ~60 |
| RP-18 | ~50 | ~60 |
| RP-18 | ~55 | ~60 |
| RP-18 | ~60 | ~60 |
| RP-18 | ~65 | ~60 |
| RP-18 | ~70 | ~60 |
| RP-18 | ~75 | ~60 |
| RP-18 | ~80 | ~60 |
| RP-18 | ~85 | ~60 |
| RP-18 | ~90 | ~60 |
| RP-18 | ~95 | ~60 |
| RP-18 | ~100 | ~60 |
| RP-18 | ~105 | ~60 |
| RP-18 | ~110 | ~60 |
| RP-18 | ~115 | ~60 |
| RP-18 | ~120 | ~60 |
| RP-18 | ~125 | ~60 |
| RP-18 | ~130 | ~60 |
| RP-18 | ~135 | ~60 |
| RP-18 | ~140 | ~60 |
| RP-18 | ~145 | ~60 |
| RP-18 | ~150 | ~60 |
| RP-18 | ~155 | ~60 |
| RP-18 | ~160 | ~60 |
| RP-18 | ~165 | ~60 |
| RP-18 | ~170 | ~60 |
| RP-18 | ~175 | ~60 |
| RP-18 | ~180 | ~60 |
| RP-18 | ~185 | ~60 |
| RP-18 | ~190 | ~60 |
| RP-18 | ~195 | ~60 |
| RP-18 | ~200 | ~60 |
| RP-18 | ~205 | ~60 |
| RP-18 | ~210 | ~60 |
| RP-18 | ~215 | ~60 |
| RP-18 | ~220 | ~60 |
| RP-18 | ~225 | ~60 |
| RP-18 | ~230 | ~60 |
| RP-18 | ~235 | ~60 |
| RP-18 | ~240 | ~60 |
| RP-18 | ~245 | ~60 |
| RP-18 | ~250 | ~60 |
| RP-18 | ~255 | ~60 |
| RP-18 | ~260 | ~60 |
| RP-18 | ~265 | ~60 |
| RP-18 | ~270 | ~60 |
| RP-18 | ~275 | ~60 |
| RP-18 | ~280 | ~60 |
| RP-18 | ~285 | ~60 |
| RP-18 | ~290 | ~60 |
| RP-18 | ~295 | ~60 |
| RP-18 | ~300 | ~60 |
| RP-18 | ~305 | ~60 |
| RP-18 | ~310 | ~60 |
| RP-18 | ~315 | ~60 |
| RP-18 | ~320 | ~60 |
| RP-18 | ~325 | ~60 |
| RP-18 | ~330 | ~60 |
| RP-18 | ~335 | ~60 |
| RP-18 | ~340 | ~60 |
| RP-18 | ~345 | ~60 |
| RP-18 | ~350 | ~60 |
| RP-18 | ~355 | ~60 |
| RP-18 | ~360 | ~60 |
| RP-18 | ~365 | ~60 |
| RP-18 | ~370 | ~60 |
| RP-18 | ~375 | ~60 |
| RP-18 | ~380 | ~60 |
| RP-18 | ~385 | ~60 |
| RP-18 | ~390 | ~60 |
| RP-18 | ~395 | ~60 |
| RP-18 | ~400 | ~60 |
| RP-18 | ~405 | ~60 |
| RP-18 | ~410 | ~60 |
| RP-18 | ~415 | ~60 |
| RP-18 | ~420 | ~60 |
| RP-18 | ~425 | ~60 |
| RP-18 | ~430 | ~60 |
| RP-18 | ~435 | ~60 |
| RP-18 | ~440 | ~60 |
| RP-18 | ~445 | ~60 |
| RP-18 | ~450 | ~60 |
| RP-18 | ~455 | ~60 |
| RP-18 | ~460 | ~60 |
| RP-18 | ~465 | ~60 |
| RP-18 | ~470 | ~60 |
| RP-18 | ~475 | ~60 |
| RP-18 | ~480 | ~60 |
| RP-18 | ~485 | ~60 |
| RP-18 | ~490 | ~60 |
| RP-18 | ~495 | ~60 |
| RP-18 | ~500 | ~60 |
| RP-18 | ~505 | ~60 |
| RP-18 | ~510 | ~60 |
| RP-18 | ~515 | ~60 |
| RP-18 | ~520 | ~60 |
| RP-18 | ~525 | ~60 |
| RP-18 | ~530 | ~60 |
| RP-18 | ~535 | ~60 |
| RP-18 | ~540 | ~60 |
| RP-18 | ~545 | ~60 |
| RP-18 | ~550 | ~60 |
| RP-18 | ~555 | ~60 |
| RP-18 | ~560 | ~60 |
| RP-18 | ~565 | ~60 |
| RP-18 | ~570 | ~60 |
| RP-18 | ~575 | ~60 |
| RP-18 | ~580 | ~60 |
| RP-18 | ~585 | ~60 |
| RP-18 | ~590 | ~60 |
| RP-18 | ~595 | ~60 |
| RP-18 | ~600 | ~60 |
| RP-18 | ~605 | ~60 |
| RP-18 | ~610 | ~60 |
| RP-18 | ~615 | ~60 |
| RP-18 | ~620 | ~60 |
| RP-18 | ~625 | ~60 |
| RP-18 | ~630 | ~60 |
| RP-18 | ~635 | ~60 |
| RP-18 | ~640 | ~60 |
| RP-18 | ~645 | ~60 |
| RP-18 | ~650 | ~60 |
| RP-18 | ~655 | ~60 |
| RP-18 | ~660 | ~60 |
| RP-18 | ~665 | ~60 |
| RP-18 | ~670 | ~60 |
| RP-18 | ~675 | ~60 |
| RP-18 | ~680 | ~60 |
| RP-18 | ~685 | ~60 |
| RP-18 | ~690 | ~60 |
| RP-18 | ~695 | ~60 |
| RP-18 | ~700 | ~60 |
| RP-18 | ~705 | ~60 |
| RP-18 | ~710 | ~60 |
| RP-18 | ~715 | ~60 |
| RP-18 | ~720 | ~60 |
| RP-18 | ~725 | ~60 |
| RP-18 | ~730 | ~60 |
| RP-18 | ~735 | ~60 |
| RP-18 | ~740 | ~60 |
| RP-18 | ~745 | ~60 |
| RP-18 | ~750 | ~60 |
| RP-18 | ~755 | ~60 |
| RP-18 | ~760 | ~60 |
| RP-18 | ~765 | ~60 |
| RP-18 | ~770 | ~60 |
| RP-18 | ~775 | ~60 |
| RP-18 | ~780 | ~60 |
| RP-18 | ~785 | ~60 |
| RP-18 | ~790 | ~60 |
| RP-18 | ~795 | ~60 |
| RP-18 | ~800 | ~60 |
| RP-18 | ~805 | ~60 |
| RP-18 | ~810 | ~60 |
| RP-18 | ~815 | ~60 |
| RP-18 | ~820 | ~60 |
| RP-18 | ~825 | ~60 |
| RP-18 | ~830 | ~60 |
| RP-18 | ~835 | ~60 |
| RP-18 | ~840 | ~60 |
| RP-18 | ~845 | ~60 |
| RP-18 | ~850 | ~60 |
| RP-18 | ~855 | ~60 |
| RP-18 | ~860 | ~60 |
| RP-18 | ~865 | ~60 |
| RP-18 | ~870 | ~60 |
| RP-18 | ~875 | ~60 |
| RP-18 | ~880 | ~60 |
| RP-18 | ~885 | ~60 |
| RP-18 | ~890 | ~60 |
| RP-18 | ~895 | ~60 |
| RP-18 | ~900 | ~60 |
| RP-18 | ~905 | ~60 |
| RP-18 | ~910 | ~60 |
| RP-18 | ~915 | ~60 |
| RP-18 | ~920 | ~60 |
| RP-18 | ~925 | ~60 |
| RP-18 | ~930 | ~60 |
| RP-18 | ~935 | ~60 |
| RP-18 | ~940 | ~60 |
| RP-18 | ~945 | ~60 |
| RP-18 | ~950 | ~60 |
| RP-18 | ~955 | ~60 |
| RP-18 | ~960 | ~60 |
| RP-18 | ~965 | ~60 |
| RP-18 | ~970 | ~60 |
| RP-18 | ~975 | ~60 |
| RP-18 | ~980 | ~60 |
| RP-18 | ~985 | ~60 |
| RP-18 | ~990 | ~60 |
| RP-18 | ~995 | ~60 |
| RP-18 | ~1000 | ~60 |

Conclusion

This study demonstrates an effective and reliable method for concentrating natural aroma compounds from acid whey using Oasis HLB, with a recovery above 90%. By reducing the retention time of the product, the recovery of 4-anisaldehyde, to 60%, was achieved. This is due to the fact that the retention times of the most concentrated aromatic compounds (benzaldehyde, 4-anisaldehyde, and vanillin aldehyde) are very similar, while benzylaldehyde exhibits the strongest retention.

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Poster Prize

The following poster presentations were awarded a poster prize:

- Best poster presentation: S. Stýblová & Berensmeier, "Concentration and Extraction of Natural 4-Anisaldehyde, 3,4-Dimethoxybenzaldehyde and Benzaldehyde From Fermented Acid Whey"
- Best Poster Prize: S. Stýblová & Berensmeier, "Concentration and Extraction of Natural 4-Anisaldehyde, 3,4-Dimethoxybenzaldehyde and Benzaldehyde From Fermented Acid Whey"