Natural flavour compounds from industrial riboflavin fermentation broths

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Introduction

Riboflavin, also known as vitamin B_2, is an important food and feed additive with an annual production of approximately 8,000 t [1]. Riboflavin is industrially produced by submerged cultivation of microorganisms in 120 m^3 fermenters.

After separation of the riboflavin containing biomass from the culture supernatant, the latter is currently considered as a waste material and is disposed. Based on the strong odour of the culture broth, a sophisticated flavour analysis was performed by means of solvent extraction and gas chromatography-mass spectrometry-olfactometry. Surprisingly, apart from some well-known lactones like γ-nonalactone and γ-decalactone, a number of uncommon lactones including e.g. γ-(Z)-dec-7-enlactone, γ-(E)-dec-5-enlactone, and γ-(Z)-dodec-6-enlactone, were detected. To unambiguously identify these compounds, a library of lactones was chemically synthesised and used as reference standards. The aroma impressions and flavour threshold values of the key compounds were determined, and an aroma extract dilution analysis (AEDA) identified γ-(Z)-dodec-6-enlactone as the main contributor to the overall aroma with a flavor dilution (FD) factor of 32,768.

Experimental design

For the AEDA, the fermentation broth was extracted with organic solvent. The concentrated extract was diluted stepwise 1+1 with solvent. Each dilution was analysed by means of GC-MS/MS-O (fig. 1). To determine the double bonds' positions in the above mentioned unsaturated lactones, a Paternò-Büchi reaction was performed. Therefore, an organic extract was purified by means of solvent assisted flavour evaporation (SAFE). The concentrated SAFE extract was further purified by means of preparative HPLC, and the Paternò-Büchi reaction was carried out with the lactone containing fractions. Afterwards, the corresponding standard compounds were synthesised by a Grignard reaction, starting with succinimide and haloalkenes.

The enantiomeric excess was determined by means of MDGC and the odour thresholds were determined by GC-FID-O equipped with an achiral and a chiral column, using (+)-γ-decalactone as internal standard.

Figure 1: gas chromatograph with tandem mass spectrometer and olfactory detection port (GC-MS/MS-O)

Results

By means of AEDA, 2-phenylethanol and γ-(Z)-dodec-6-enlactone were identified as the compounds with the highest FD factor (32,768).
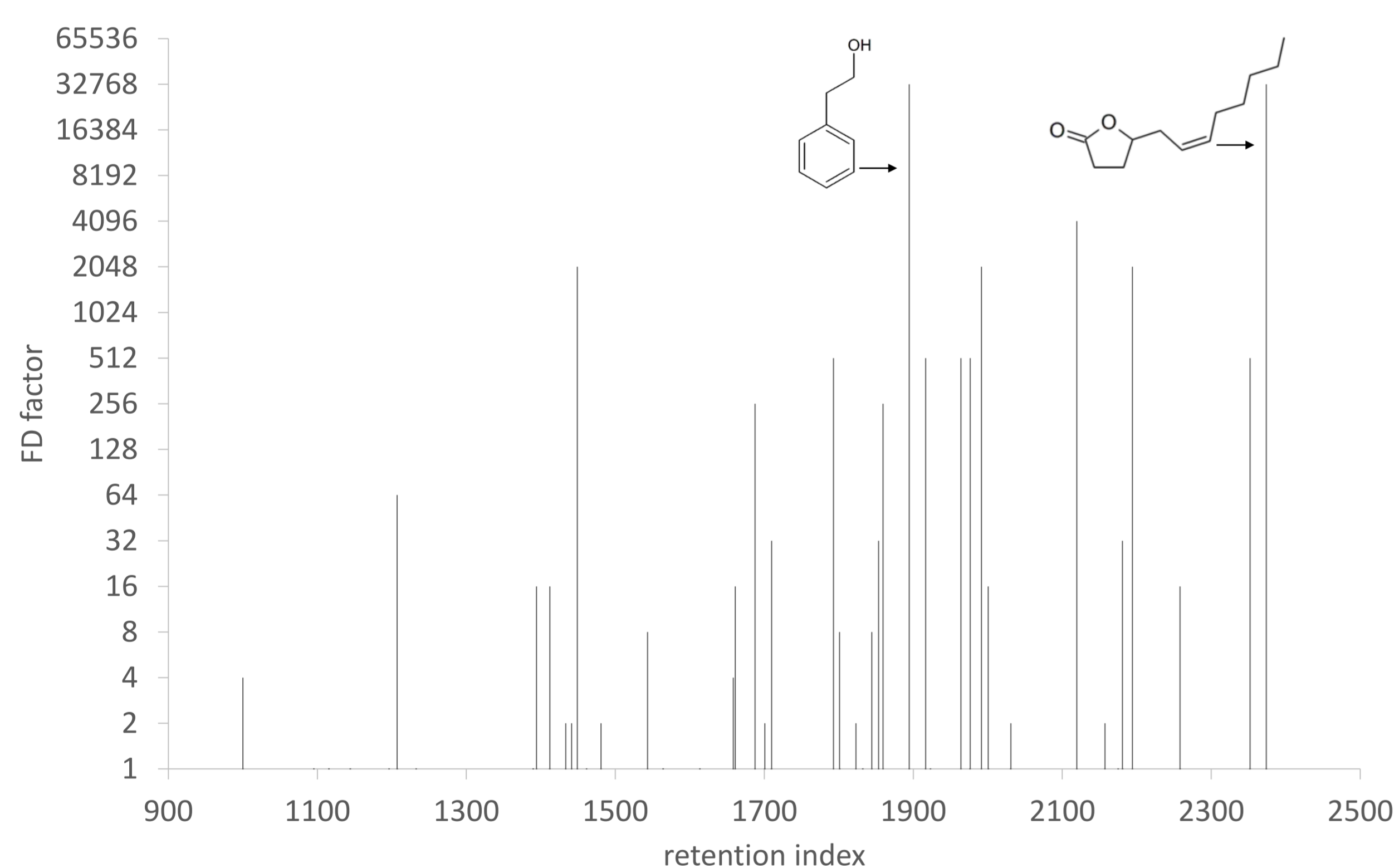

Figure 2: FD chromatogram of the AEDA of the riboflavin fermentation broth

The enantiomeric excess was determined by means of MDGC. The quantitation was performed by means of GC-FID after liquid/liquid extraction (tab. 1).

The Paternò-Büchi reaction showed fragments that suggested a double bond position in the C_{10} lactones at position 5 and 7, respectively, and at position 6 in the C_{12} lactone. Therefore, the corresponding compounds were synthesised. The comparison of the retention indices and the mass spectra confirmed that the detected unsaturated lactones were γ-(Z)-dec-7-enlactone, γ-(E)-dec-5-enlactone, and γ-(Z)-dodec-6-enlactone (fig. 3).
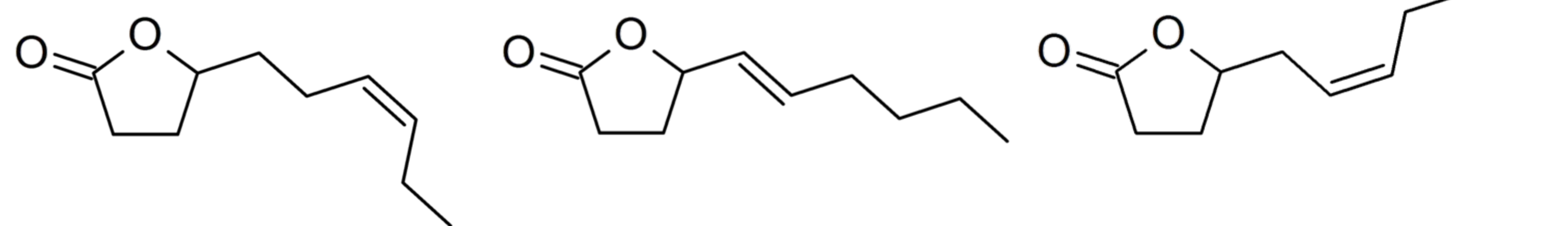

Figure 3: unsaturated lactones found in the fermentation broth;
left: γ-(Z)-dec-7-enlactone; middle: γ-(E)-dec-5-enlactone; right: γ-(Z)-dodec-6-enlactone

After the synthesis of the lactones, the odour thresholds of the racemates were determined by means of GC-FID-O equipped with an achiral column. Subsequently, the odour thresholds of the enantiomers were also determined by means of GC-FID-O, equipped with a chiral column (tab. 2). First, the odour threshold of (+)-γ-decalactone was determined using (E)-2-decenal as internal standard. Second, (+)-γ-decalactone was used as internal standard for the determination of the odour thresholds of the remaining lactones (racemates and enantiomers).

Table 1: Enantiomeric excess and concentration in the fermentation broth

compound	enantiomeric excess	concentration [mg L^{-1}] (σ)
γ-nonalactone	1.2	2.0 (0.2)
γ-decalactone	72.1	75.7 (2.1)
γ-(Z)-dec-7-enlactone	84.5	4.4 (0.2)
γ-(E)-dec-5-enlactone	29.4	1.5 (0.1)
γ-dodecalactone	15.1	36.9 (1.3)
γ-(Z)-dodec-6-enlactone	48.0	46.8 (1.0)

Table 2: Odour thresholds (ng L^{-1}) in air of the racemic lactones and the enantiomers

compound	racemate	R enantiomer	S enantiomer
γ-nonalactone	0.68	0.62	0.62
γ-decalactone	0.74	0.30	0.61
γ-(Z)-dec-7-enlactone	2.90	3.36	3.36
γ-(E)-dec-5-enlactone	1.35	1.26	0.63
γ-dodecalactone	0.32	0.16	0.31
γ-(Z)-dodec-6-enlactone	0.06	0.13	0.13

Conclusions

This fermentation process represents a novel and sustainable way for the biotechnological production of highly potent aroma compounds. Although biological sources for R-configured saturated lactones exist, there are only a few known for the production of the S-configured lactones. Additionally, unsaturated lactones are barely commercially available.

Literature:
[1] Ledesma-Amaro et al. *Metabolic engineering of riboflavin production in* Ashbya gossypii *through pathway optimization* Microb Cell Fact (2015)