Institut für Sicherheit und Qualität bei Getreide



Are micro-organisms settling on rapeseed responsible for sensory bad quality virgin rapeseed oil?

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Introduction

Sometimes virgin rapeseed oil is characterized by an unpleasant perception for musty or fusty off-flavor strongly impairing the quality of the product. This impression is associated with the occurrence of higher amounts of certain volatile aroma compounds such as 2- and 3-methylbutanal, acetoin and others. It has not yet been investigated whether these compounds can result from the metabolism of microorganisms that colonize rapeseed.

Aim

In the present work the profile of volatile compounds formed as result of a microbial infestation of rapeseed should be

investigated to identify compounds also found in virgin rapeseed oil with unpleasant sensory characteristic.

Workflow

Isolation and cultivation of bacteria from different rapeseeds on rapeseed meal agar as growing medium (10 g/L rapeseed meal, 14 g/L agar-agar, 0,4 g/L CaCl₂·2H₂O)

Extraction of total genomic DNA and determination by Sanger sequencing \rightarrow Identification by comparison with GenBank database

Olfactometric selection of interesting bacterial strains

Cultivation of selected strains in headspace vial on rapeseed meal agar (5 days at 28 °C)

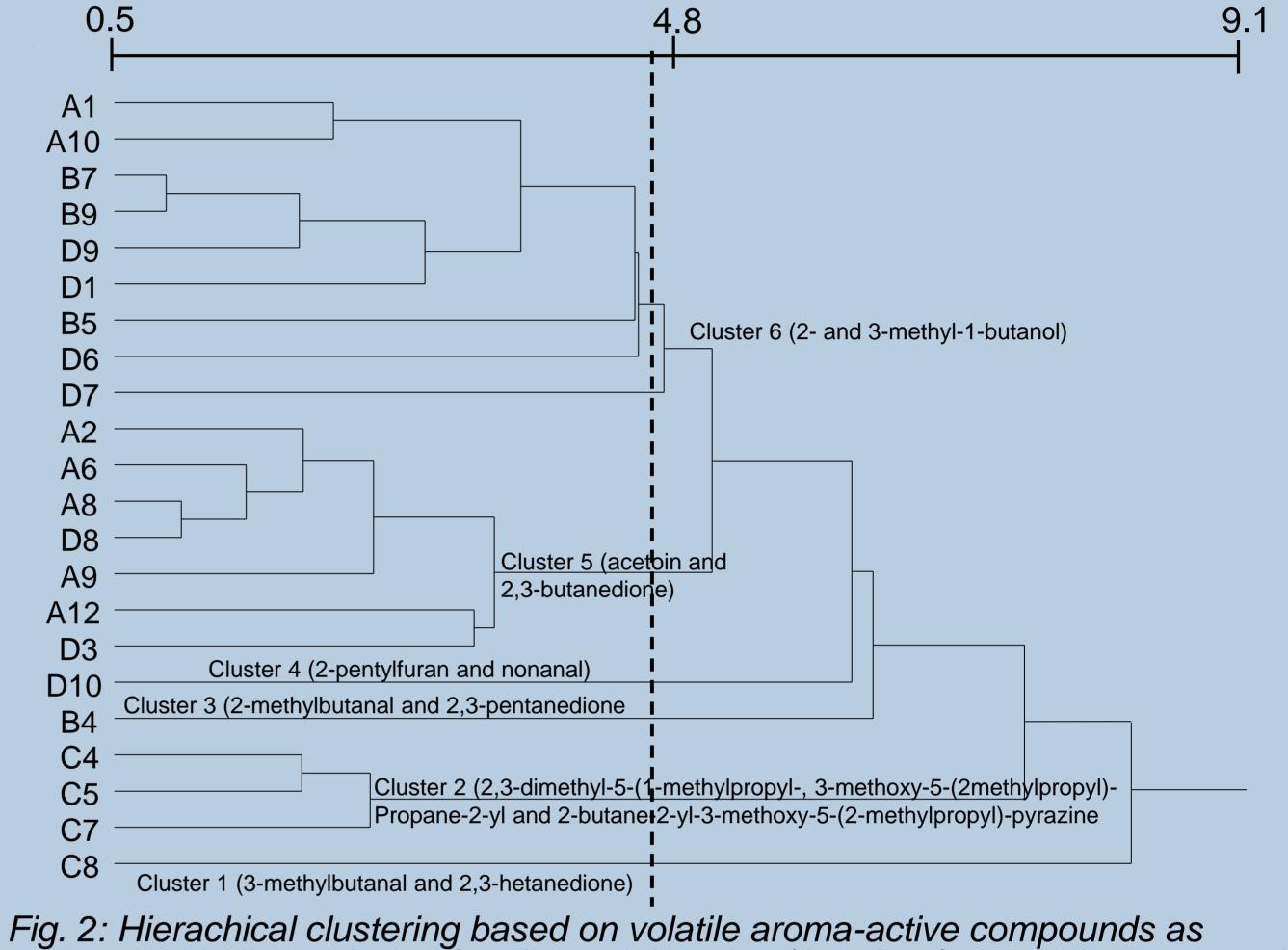
> Analysis of volatile compounds by SPME-GC-MS (extraction of the volatile compounds: 60 min at 60 °C)

> > Comparison with results from the literature

Fig. 1: Experimental approach for isolation of relevant bacteria strains and analysis of volatile compounds.

Tab. 1: 22 bacterial strains with sensory perceptions of typical rapeseed oil off-flavours

Code	Identified bacteria ^a	Total smell intensity ^b	Attributes most often mentioned
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metabolic products of 22 bacterial strains (A1 - D10) populating rapeseed. Cluster 1 - 6 show bacterial strains with similar volatile profiles.

A1	Stenotrophomonas rhizophila	6	musty, wet seeds
A2	Paenibacillus sp.	10	fusty, sour
A6	Erwinia amylovora	4,5	fusty, sour
A8	Pseudomonas sp.	4,5	fusty
A9	Erwinia sp.	5,5	musty, fusty
A10	not identified	4	musty, fusty
A12	not identified	8	musty, fusty, faecal
B4	Paenibacillus sp.	4	sweety, sour
B5	not identified	11	musty, fusty, mushroom-like
B7	Paenibacillus sp.	5	cheese-like, fusty
B9	not identified	6	sour, fusty
C4	not identified	4	musty
C5	Pseudomonas sp.	6,5	musty, sour, aromatic
C7	Sphingobacterium sp.	10	cheese-like, meaty, aromatic
C8	not identified	17	scruffy, fusty, cheese-like
D1	Pantoea agglomerans	6	musty, aromatic
D3	Stenotrophomonas sp.	11	fusty, cheese-like
D6	Pseudomonas sp.	6,5	musty, fusty
D7	not identified	12	fusty, meaty
D8	not identified	5	cheese-like, fusty
D9	Pseudomonas sp.	7	fusty
D10	Pseudomonas sp.	18	noticeable different, fermented

^aGenera or rather species of 46 bacterial strains isolated from rapeseed and identified by Sanger sequencing and GenBank database comparison of determined DNA sequences. ^bsummed intensity of the sensory fault detected by four of six panelists.

Tab. 2: Volatile aroma-active compounds derived from the 22 bacteria strains of Table 1 cultivated on rapeseed meal agar. Highlighted compounds were also found and described as aroma-active in virgin rapeseed oils

No.	Compound	RI	Aroma attribute
1	dimethyl sulfide	545	cabbage
2	2,3-butanedione	562	butter
3	2-methylfuran	570	ethereal
4	3-methylbutanal	595	malty
5	2-methylbutanal	602	cacao, almond
6	2-pentanone	621	ethereal, fruity
7	2,3-pentanedione	629	cream, butter
8	acetoin	641	cream, butter
9	3-methyl-1-butanol	671	malty
10	2-methyl-1-butanol	676	onion
11	dimethyl disulfide	680	onion, cabbage, foul
12	2,3-heptadione	839	fruity, butter, oily
13	2-methylbutanoic acid ethyl ester	851	fruity
14	Tricyclene	920	flowery
15	2-pentylfuran	990	green beans
16	nonanal	1104	lemon, soapy
17	2,3-dimethyl-5-(1methylpropyl)-pyrazine	1185	nutty
18	3-methoxy-5-(2-methylpropyl)-2-propane-2-ylpyrazine	1333	а
19	2-butane-2-yl-3-methoxy-5-(2-methylpropyl)-pyrazine	1384	а
adescr	ibed as intense with no further specification		

Results and Discussion

> Bacteria identified on rapeseed by DNA sequencing (Sanger sequencing) and GenBank database comparison of determined DNA sequences:

Stenotrophomonas rhizophila, Stenotrophomonas sp., 4 Paenibacillus spp., Pseudomonas fluorescens, 2 Pseudomonas spp., Erwinia amylovora, Bacillus spp., Pantoea agglomerans, 33 further strains (not identified) (Tab. 1).

- > The smell of 22 bacteria strains was characterized olfactometrically as similar to the typical off-flavor of virgin rapeseed oil (Tab. 1).
- > 19 aroma-active compounds have been identified as metabolic products from 22 bacterial strains shown in Table 1 after cultivation on rapeseed meal agar (Tab. 2).
- > Acetoin, 2-methylbutanoic acid ethyl ester, 3-methylbutanal were also found in sensory bad virgin rapeseed oil, dimethyl sulfide, 2-methylbutanal are also described with aroma values > 1 in the literature; nonanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-pentylfuran were also found in virgin rapeseed oil (Tab. 2).
- > Clustering of bacterial strains resulted in six groups (clusters) with similar profile of volatile compounds (Fig. 2).

Conclusion

> Micro-organisms populating on rapeseed during storage may strongly influence the sensory quality of virgin rapeseed oil by the development of volatile aroma-active metabolic products.

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