FORMATION OF MOUSY OFF-FLAVOUR
IN WINE BY LACTIC ACID BACTERIA

by

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THESIS SUMMARY

Formation of mousy off-flavour in wine by lactic acid bacteria.

Mousy off-flavour is an infrequent yet serious spoilage phenomenon in wine and other fermented beverages, which is commonly associated with the growth and metabolism of certain lactic acid bacteria (LAB) and the spoilage yeast Dekkera / Brettanomyces. Two compounds known to cause the characteristic and offensive mousy-like off-flavours are the N-heterocyclic volatile bases 2-acetyltetrahydropyridine (ACTPY) and 2-ethyltetrahydropyridine (ETPY). Since there is no satisfactory method for the removal of mousy off-flavour, this spoilage can invoke substantial economic loss to the wine producer. The aims of this thesis were to investigate the following aspects of the formation of mousy off-flavour by wine LAB:

1. Development of a sensitive and reliable procedure for the quantification of N-heterocyclic compounds causing mousy off-flavour in wine;
2. Survey the abilities of wine LAB and other wine bacteria to produce mousy off-flavour and the causative mousy compounds;
3. Investigate the substrates and metabolism of mousy compound formation by LAB.

Difficulties were encountered in the analysis of ACTPY due to its chemical and chromatographic instability, suggesting why previous research efforts have failed to quantify mousy compounds. Of several procedures assessed for the reliable extraction and quantification of low concentrations (µg/L level) of mousy compounds, a continuous liquid - liquid extraction (CLLE) method was developed and used in association with gas chromatography - mass spectrometry (GC-MS). The CLLE / GC-MS method was validated by demonstrating efficient and artefact-free recovery of mousy compounds from spiked Riesling wine. Using this procedure, three structurally related compounds, ACTPY, ETPY and a newly discovered and highly potent N-heterocycle, 2-acetyl-1-pyrroline (ACPY), were found to be unique components of mousy wines. Of the three mousy compounds, ACTPY was the most common and occurred at the highest concentration (106 µg/L), whereas ACPY and ETPY occurred less frequently and at maximum concentrations of 7.8 and 4.5 µg/L, respectively. The mousy aroma properties of ACPY were confirmed by GC-sniff analysis.

Thirty five LAB were screened for the ability to produce mousy off-flavour by a qualitative alkaline test strip procedure. In addition to Lactobacillus brevis and L. cellobiosus, which were known to be associated with mousy off-flavour, a diversity of LAB species, particularly heterofermentative Lactobacillus spp. and Oenococcus oeni, exhibited this ability in a range of ethanolic and wine-based media. The homofermentative Pedioococcus spp., however, were generally lacking in this ability.

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Selected wine bacteria were tested for the production of mousy compounds in nutritionally complex (Carr-MEI) and chemically defined (S1) media. In Carr-MEI medium, strains of Lactobacillus spp., O. oeni, Pediococcus spp. and Gluconobacter oxydans each produced one or more of ACTPY, ACY and ETPY generally in the concentration range of 0.1 to 30 µg/L. Exceptionally high concentrations of ACTPY (259 µg/L) were produced by the type strain L. hilgardii DSM 20176. In contrast, synthetic (S1) medium supported only limited production of mousy compounds, despite similar growth characteristics to these with Carr-MEI medium.

The metabolism of mousy compounds by LAB was studied utilising a high cell density incubation (HCDI) technique with a basal assay (BA) medium, the main components of which were D-fructose (50 g/L), ethanol (5% v/v), L-lysine (5 g/L), L-ornithine (5 g/L), metal salts and organic acids. Essential substrates of ACY and ACTPY formation by L. hilgardii DSM 20176 were the availability of a fermentable carbohydrate (e.g. D-fructose), ethanol and iron (ferrous sulfate). In addition, L-ornithine stimulated the formation of ACTPY, whereas L-lysine stimulated the formation of ACTPY and repressed ACY. The formation of ETPY, however, was little influenced by the availability of carbohydrate, L-ornithine or L-lysine. Other nutritional factors found to affect the formation of ACY and ACTPY by L. hilgardii DSM 20176 in BA medium included the presence of malic acid and acetaldehyde, and the source of carbohydrate and amino acid. Replacement of ethanol with α-propanol led to the formation of propionyl-tetrahydropryrazine, although this reaction did not occur with iso-propanol. The incorporation of deuterated ethanol (d6-ethanol) into the acetyl side chain of ACTPY and ACY, and of deuterated acetaldehyde (d4-acetaldehyde) into the acetyl side chain of ACTPY, confirmed that ethanol and acetaldehyde were precursors of these mousy compounds. These results also suggested that the attachment of the carbonyl side chain involved prior reduction of a primary alcohol to the corresponding aldehyde.

A pathway for the formation of ACY and ACTPY by heterofermentative LAB is proposed. In this scheme, the co-metabolism of exogenous carbohydrate and ethanol force the accumulation of C-2 intermediates of the heterolactic fermentation of sugars (e.g. acetyl-coenzyme A). These C-2 compounds may then concurrently acetylate N-heterocyclic intermediates of L-ornithine and L-lysine metabolism, thus leading to the production of ACY and ACTPY.
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