PRODUCTION AND LOCALISATION OF HAZE
PROTECTIVE MATERIAL FROM SACCHAROMYCES
CEREVISIAE

by

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A thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

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June 1997
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ABSTRACT

The effectiveness of methods to extract haze protective material (HPM) from whole yeast cells was tested on three winemaking *Saccharomyces cerevisiae* strains [Maurivin Prise de mousse (PDM); flocculent yeast AWRI 65; Champagne yeast AWRI 85]. For the three strains, hot extraction with SDS (boiling cells in Tris buffer for 5 min) and the full zymolyase treatment (which combined a pretreatment of the cells with DTE and EDTA followed by an enzymatic digestion of the cell debris with zymolyase) were the most efficient in releasing HPM. In contrast, hot citrate extraction (using an autoclave) was far less effective whereas disruption of the cells using a French press device released haze-forming mannanproteins. Overall, better results were obtained with Maurivin PDM and AWRI 85 compared to AWRI 65.

Thorough studies of the parameters responsible for HPM release from Maurivin DPM showed that SDS itself was not necessary to extract HPM. Boiling the cells in Tris buffer, without SDS, efficiently extracted HPM. Similarly, the decomposition of the full zymolyase treatment showed that HPM was more specifically extracted during the pretreatment rather than during the enzymatic digestion with zymolyase. Further investigation revealed that HPM was released by EDTA whereas DTE preferentially extracted haze forming mannanproteins. The implications of these data for the location of HPF and its interactions with other cell wall components are discussed.

The extracellular material released during fermentation of Maurivin PDM yeast during storage on yeast lees was recovered either by ultrafiltration or ethanol precipitation. In both cases, the material collected was active in reducing the haze without the enrichment of the extract in mannanproteins being necessary.

Antibodies to a purified wine HPF were used to localise HPF in Maurivin PDM yeast cell. No cross-reactivity against yeast mannan or invertase was detected by the methods of gel double diffusion and western immunoblotting. Agglutination assay indicated that HPF was present on the cell wall surface. Successful immunogold labelling followed by transmission electron microscopy showed HPF to be distributed on the cell wall surface, in particular, at the periphery and innermost layers of the cell wall.