Expression and production of the *Saccharomyces cerevisiae* haze protective factor 2 for sensory studies and further investigation into the role of glycosylation

by

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THESIS SUMMARY

White wine clarity is essential, but it can be marred by the presence of a protein haze. This protein haze is predominantly formed by grape-derived thaumatin-like proteins and chitinases, which can slowly denature and aggregate if left in bottled wines.

Currently bentonite fining is used by the wine industry to prevent protein haze. Bentonite consists of fine clay particles that, when added to wine, bind and remove the haze-forming proteins. However this method is inconvenient, time-consuming, and causes significant losses of wine. It is estimated that this process costs the Australian wine industry $50 m annually in wine losses alone. Alternatives are thus being investigated.

The principal objective of this thesis was to investigate the sensory effects on wine of an alternative method to bentonite fining: addition of haze protective factor 2, known as Hpf2. Hpf2 is a *Saccharomyces cerevisiae* mannoprotein that has been shown to reduce protein haze in wines. It is a highly mannosylated 180 kDa protein, of which approximately 75% by weight is mannose. Previous work has shown that the addition of approximately 200 mg L^{-1} Hpf2 to wines reduces the visible haze in wine by approximately 50%.

Hpf2 is naturally present in wines at concentrations of less than 10 ng L^{-1}, much lower than the concentration required for haze protection activity. However, the sensory impacts involved with the addition of such high concentrations of Hpf2 in wine have never been studied. This knowledge is essential for the future commercial prospects of this alternative approach to protein stabilisation of wine.

To undertake sensory studies, over 1 g of Hpf2 would be required. Presently, the laboratory-scale process for the production of a 6-histidine tagged version of the protein, 6xHisHpf2, in a laboratory yeast strain of *S. cerevisiae*, produces only milligram quantities. Consequently, the first challenge of this research was to scale up the existing process to produce sufficient quantities of Hpf2.

The first attempt to increase the production level was by over-expression in the bacteria *Escherichia coli*. Although several approaches were trialled, 6xHisHpf2 was unable to be successfully and consistently expressed in this system. The second method was by improving the original yeast expression system, and the expression level was able to be improved approximately 10-fold. This improved expression method was scaled up to produce and then purify over 1 g of protein. Several quantification methods were assessed to determine the efficiencies of each purification step, with slot blot analysis proving successful.

Sensory trials were conducted to establish the effect of 6xHisHpf2 on wines, with duo-trio studies conducted assessing both aroma and palate of the wines. Invertase, another yeast haze protective factor, was also trialled. It was found that the addition of an active level of 6xHisHpf2 or invertase did not cause a significant difference in the aroma or palate of wines.

In addition to this main study, the role of the glycosylation was studied. 6xHisHpf2, produced in a different yeast, *Pichia pastoris*, was found to be 83 kDa, with only 50% mannose. This protein was compared to the *S. cerevisiae* protein in its ability to reduce protein haze, and it was shown that the *P. pastoris* protein could reduce haze, but not as effectively as the *S. cerevisiae* protein.

The finding that Hpf2 does not affect the sensory properties of wine is essential if Hpf2 is to be used commercially, as winemakers and wine consumers would most likely reject an additive that alters the wine aroma or palate. This work has brought the wine industry a step closer to a new method for protein haze prevention in white wines.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available in all forms of media, now or hereafter known.

Oenone Jean Macintyre
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ABBREVIATIONS

6xHis	six consecutive histidine amino acids
AWRI	Australian Wine Research Institute
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BSA	bovine serum albumin
CDGJM	chemically defined grape juice medium
Da	Dalton
DNA	deoxyribonucleic acid
DDM	Delft defined media
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
GM	genetically modified
GPI	glycosylphosphatidylinositol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPF	Haze protective factor
HPLC	high performance liquid chromatography
IMAC	immobilised metal affinity chromatography
IPTG	isopropyl β-D-thiogalactopyranoside
kDa	kilo Dalton
LB	Luria-Bertani
LDM	Lund defined media
MW	molecular weight
NBT	nitro blue tetrazolium
Ni-NTA	nickel-nitrilotriacetic acid
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pI	isoelectric point
PMI	phosphomannose isomerase
PNGase F	peptide-*N*-(acetyl-β-glucosaminyl) asparagine amidase
RNA	ribonucleic acid
SCM	synthetic complete medium
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SE	standard error
TAE	Tris-acetate-EDTA
TBS	Tris buffered saline
TBS-T	Tris buffered saline with Tween 20
TCA	trichloroacetic acid
TFA	trifluoroacetic acid
tRNA	transfer ribonucleic acid
Tris	tris(hydroxymethyl)aminoethane
V	volts
WCE	whole cell extract
YPD	yeast peptone extract