

**A MULTIPRONGED APPROACH TO ENCOURAGING
PROLINE UTILISATION BY WINE YEAST**

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Abstract

Deficiency of assimilable nitrogen for *Saccharomyces cerevisiae* can cause stuck or sluggish fermentations, which are common problems in the wine industry. Supplementation strategies are usually employed by winemakers to avoid such problems. However, this practice can give undesirable outcomes, which negatively influence wine quality. Enhancing the use of nitrogen sources already in grape juice represents a possible solution to this problem.

Proline is the predominant amino acid in grape juice, but only a small quantity is utilised during fermentation, initially because proline uptake is repressed in the presence of preferred nitrogen sources. Once these nitrogen sources have been exhausted, oxygen is no longer available for the activity of proline oxidase, the enzyme responsible for the first step in proline catabolism.

This study aimed to develop yeast strains capable of proline utilisation, so as to overcome nutrient-related fermentation problems, thereby improving fermentation reliability and overall wine quality. It also sought to evaluate winemaking techniques that exploit the abundance of proline in grape juice as a nitrogen source, i.e. the use of micro-oxygenation during fermentation to enhance proline utilisation.

A convenient and economical spectrophotometric method using isatin as a derivatising agent for proline determination was developed for use in the first study.

This method was found to be robust for the analysis of both fermentation samples and table wines. Subsequently, two novel yeast strains, Q2 and Q7, that were capable of utilising proline constitutively (i.e. in the presence of preferred nitrogen sources) were generated by ethyl methanesulfonate (EMS) mutagenesis. Intracellular proline determined by the isatin colorimetric method suggested that the isolates utilised proline more efficiently during fermentation compared to the wild type strain, QA23. Quantitative real-time polymerase chain reaction (QRT-PCR) results showed that expression of key genes involved in proline degradation and transportation were altered in Q2 and Q7. Experiments involving micro-oxygenation strategies were subsequently carried out with Q2 and EC1118 to evaluate the impact on proline utilisation. A range of oxygenation rates and addition times were evaluated during model fermentations to assess their impact on proline utilisation. Fermentations with 8 mg/L dissolved oxygen resulted in significantly reduced fermentation times (by about 50 hours) compared to the un-oxygenated control, which took about 365 hr. About 220 mg/L more proline was consumed when oxygen was added during the cell growth and stationary phases compared to control (anaerobic) conditions. Interestingly, for yeast strain comparison, Q2 was found to be a higher glycerol producer, but further research is required to confirm these results during fermentation of grape juice.

This study suggests that proline can be exploited as an alternative nitrogen source when fermentation is undertaken with certain novel yeast strains and/or with

oxygen addition. It is worth noting that in this study, the tested strains Q2 and EC1118 expressed different oxygen requirements for improved proline utilisation. Different strains are required for different wine making conditions, thus the impact of oxygen addition must therefore be considered carefully.

List of abbreviations

Abbreviation	Full term
ARG	Arginase
Arg	Arginine
Asn	Asparagine
CDGJM	Chemically defined grape juice media
Cys	Cysteine
DAP	Diammonium phosphate
EMS	Ethyl methanesulfonate
GABA	γ -aminobutyrate
GK	γ -glutamyl kinase
Glu	Glutamic acid
Gly	Glycine
GPR	γ -glutamyl phosphate reductase
GSH	Gluthathione
His	Histidine
hr	Hour
Leu	Leucine
Lys	Lysine
Met	Methionine
NCR	Nitrogen catabolite repression
ORF	Open reading frame
OTA	Ornithine transaminase
P5C	Δ 1-pyrroline-5-carboxylate
P5CDH	P5C dehydrogenase
Phe	Phenylalanine
PO	Proline oxidase
Pro	Proline
PROVEAN	Protein variation effect analyser
Ser	Serine
Thr	Threonine
Tyr	Tyrosine
Val	Valine
YAN	Yeast assimilable nitrogen
YEPD	Yeast extract peptone dextrose