BIOTECHNOLOGY APPLICATIONS in winemaking industry

Sara Torrent Vergés

Biotecnology grade - Universitat Autònoma 08193 Bellaterra - Catalonia, Spain.

1. Introduction

Biotechnology applications in winemaking industry are related to improve economical aspects of the process and to enhance the quality of the final product in order to satisfy the consumer. Currently, wines with less alcohol content, wines without biogenic amines or good quality wines at every price point are more demanded. Scientific advancements in the lasts decades, as well as recombinant DNA techniques development have made possible the creation of new tools to achieve these challenges. The main techniques employed by large-scale wineries are the addition of dried enzymes and yeasts solutions in specific points during the winemaking process.

2. The winemaking process

Unloading → **Stemming and crushing** → **Maceration and pressing** → **Alcoholic fermentation and maturation** → **Clarification** → **Stabilization** → **Bottling**

Only for white wines. Red and rosé wines are macerated while fermented.

Fermentation lasts 15-20 days for white wines and between 15 days and 3 weeks for red ones (Fermentation +Maceration). Some wines are submitted to a second malolactic fermentation.

By a tangential filter

1. Tartaric acid precipitation
2. Sulfite addition
3. Filtration

Pictures taken from *Bodegues Nuviana*, Lleida.

3. Practices to improve the winemaking process

HOW CAN THIS PROCESS BE IMPROVED?

BIOTECHNOLOGICAL METHODS

DRY ENZYME ADDITION

Production methods

Aspergillus spp.

Tricchoderma spp.

Sorts of enzymes

Enzyme	Process or product improvement
β-glucanase	Clarification
Proteolytic E	Stabilization (during aging)
Laccases	Stabilization (pre fermentative)
Glycosidases	Aroma
Pectolytic E	Clarification, wine's color

- The most widely used.
- Usually added during crushing.
- Act on pectins (found in grape's cell walls)

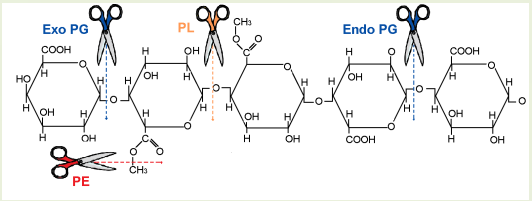

Figure 1. Pectin structure and the enzymes contained in employed preparations to hydrolyze it: **PE (pectinesterase), Exo and Endo PG (exo and endo polygalacturonase)** and **PL (pectinlyase)**.

Table 1. Effect of pectin enzyme treatment on juice yield and clarity after clarification for several grape varieties (Ough and Crowell, 1979). Juice yield is enhanced with enzyme treatment which can be related with greater anthocyanin extraction and enhanced total yield. The clarification is also improved as the packed solids are reduced after this step when receiving the enzyme treatment.

	Juice yeld after pressing (%)		After racking and centrifuging			
			Packed solids (%)		*Clarity*	
	No E	E	No E	E	No E	E
Emerald Riesling	54.9	68.6	6.0	0.5	Dull	Brilliant
Palomino	55.1	59.2	0.1	0.1	Dull	Clear
Sauvignon blanc	66.4	72.0	0.4	0.2	Dull	Clear
Tokay	57.0	60.0	0.6	0.1	Clear	Brilliant

DRY YEAST ADDITION

Obtention methods

Yeast breeding

Techniques

- **Variant selection**
- **Hybridization**
- **Mutagenesis**

Techniques for specie and strain selection

- Sequencing of rDNA genes
- RFLP of rDNA and mtDNA
- Quantitative PCR
- PCR-DGGE
- Microsatellites

Genetic engineering

Techniques

- Transformation
- Plasmid vectors
- Expression and secretion *cassettes* for the expression of encoded genes.

Targets

- Improved fermentation performance
- Improved efficiency
- Improved control of spoilage bacteria
- Improved wine flavor and aroma

- Improved wine wholesomeness

ECM001 (genetic engineered *Saccharomyces cerevisiae* strain)

- Reduces ethyl carbamate formation in wine which is considered a suspected human carcinogen.

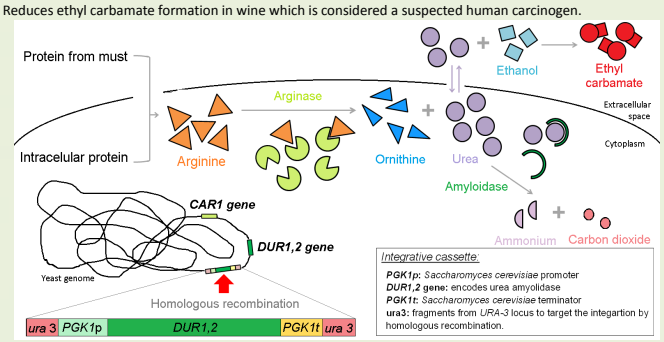

Figure 2. Mainly due to arginine degradation pathway, urea is accumulated within the yeast cell. It can be degraded to useful nitrogen sources by the amyloidase enzyme. However, arginine conversion into urea is faster than urea degradation so, it is excreted outside the cell and re-absorbed later. While being outside the cell, urea can combine with ethanol producing ethyl carbamate (urethane). ECM001 strain contains another copy of the *DUR1,2* gene (red arrow), from a *Saccharomyces cerevisiae* strain (self-cloning), thus, enhancing amyolidase amount within the cell and reducing ethyl carbamate production.

Table 2. Production of ethyl carbamate in Chardonnay wine produced with UC Davis 522 strain. (Coulon J, Husnik J. Et al , 2006). It shows a reduction of 89,1% of ethyl carbamate.

	UC Davis 522 WT	UC Davis 522* (containing the integration *cassette)*
Ethyl carbamate production (µg/L)	87,85	9,61

4. Conclusions

The employment of dried enzymes and yeasts solutions has facilitated winemaking for large producing wineries. In the United States, two genetically modified yeast strains had already been brought onto the market, whereas in Europe these techniques are mainly limited to yeast breeding, selection and isolation. According to **2001/18/CE** directive and **(EC) 1829/2003** and **(EC) No 1830/2003** regulations, genetically modified yeasts for wine production are allowed in Europe and wine does not need to be labeled since yeasts are removed from the manufactured product. However, there is a skeptic attitude towards GMO which cause difficulties in the introduction of these innovative methods. Self-cloning techniques (used in ECM001 strain generation) would be a better accepted alternative, however is still far from being used with a commercial use in Europe.

References: Ough, C.S.; Crowell, E.A. *Pectic enzyme treatment of white grapes: temperature, variety and skin-contact time factors.* Am.J.Enol.Vitic. 1979, 30 (1), 22-27

Coulon J.; Husnik J.I. *et al. Metabolic Engineering of Saccharomyces cerevisiae to Minimize the Production of Ethyl Carbamate in Wine.* Am.J.Enol.Vitic. 2006, 57 (2), 113-124