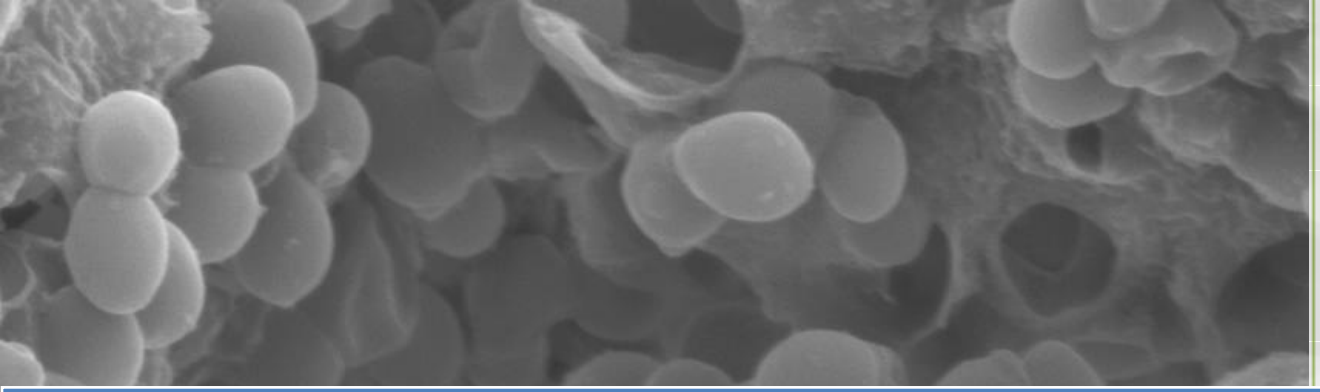


infowine 2012

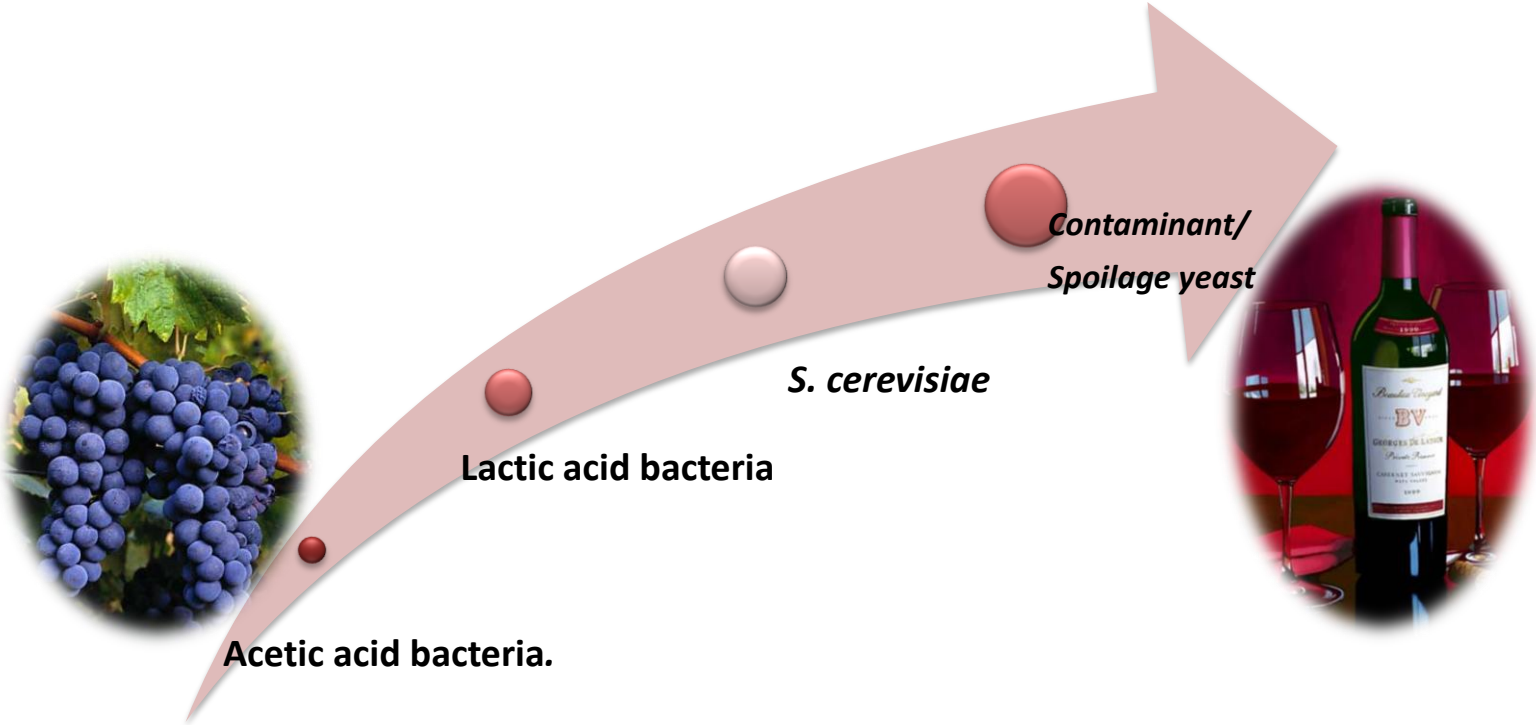
Reduction of wine volatile acidity by immobilized *S. cerevisiae* cells in alginate-chitosan beads

Alice Vilela[1]
Dorit Schuller[2]
Arlete Mendes-Faia[1]
Manuela Côrte-Real[2]

[1] (IBB/CGB-UTAD), Universidade de Trás-os-Montes e Alto Douro, 5001-801 Vila Real, Portugal.
[2] Centre of Molecular and Environmental Biology, Dep of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

Volatile acidity…an enological problem

- Acetic acid is the main component of volatile acidity, and critical for wine quality;
- Above 0.8 – 0.9 $g.l^{-1}$, acetic acid has a detrimental organoleptical effect (acidic wine).



Available solutions?

Stabilization followed by mixture with other wines

Distillation

Nanofiltration and Reverse osmosis

"Remostagem" or refermentation

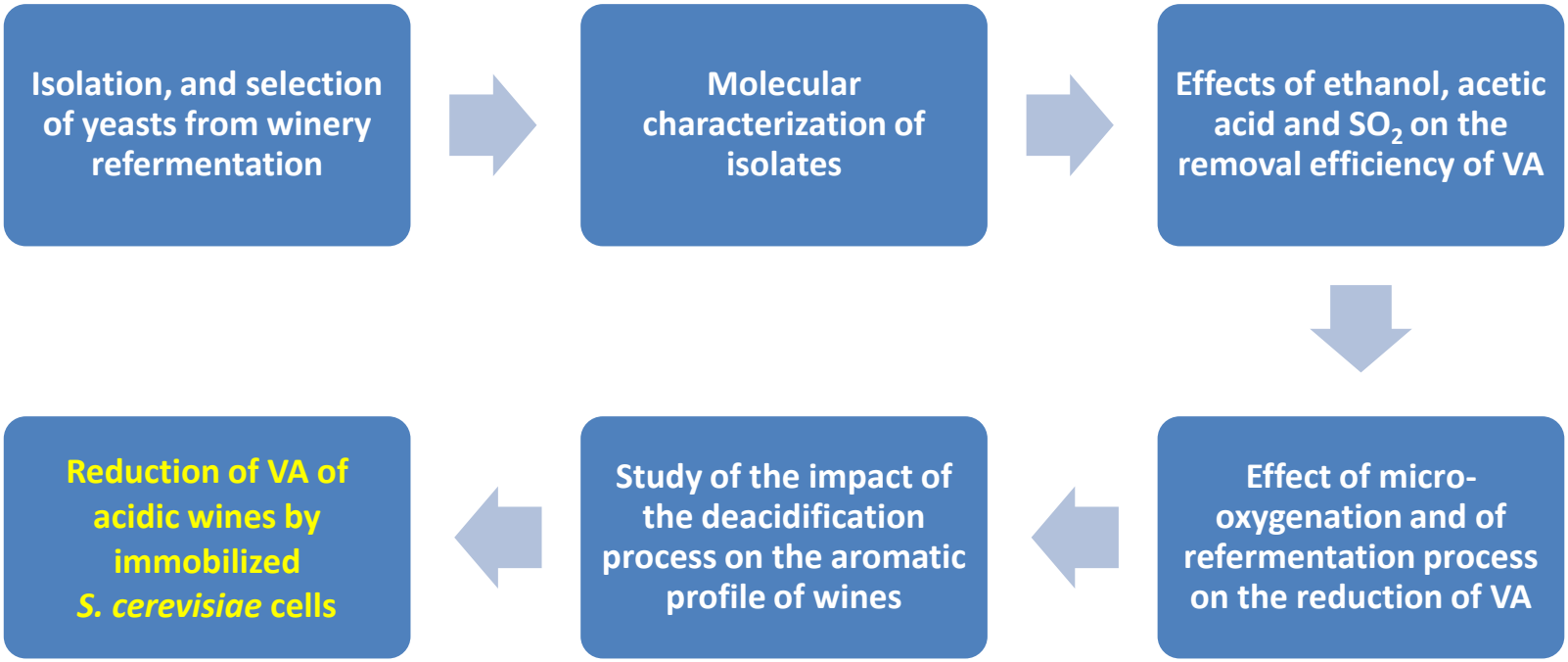
Refermentation

- "…one third of an acidic wine is mixed with two thirds of freshly crushed grapes or of the residual marc from the fermentation of a finished wine (remaining pulp, after draining the newly made wine)."

Ribéreau-Gayon *et al*., 1975

Main goal

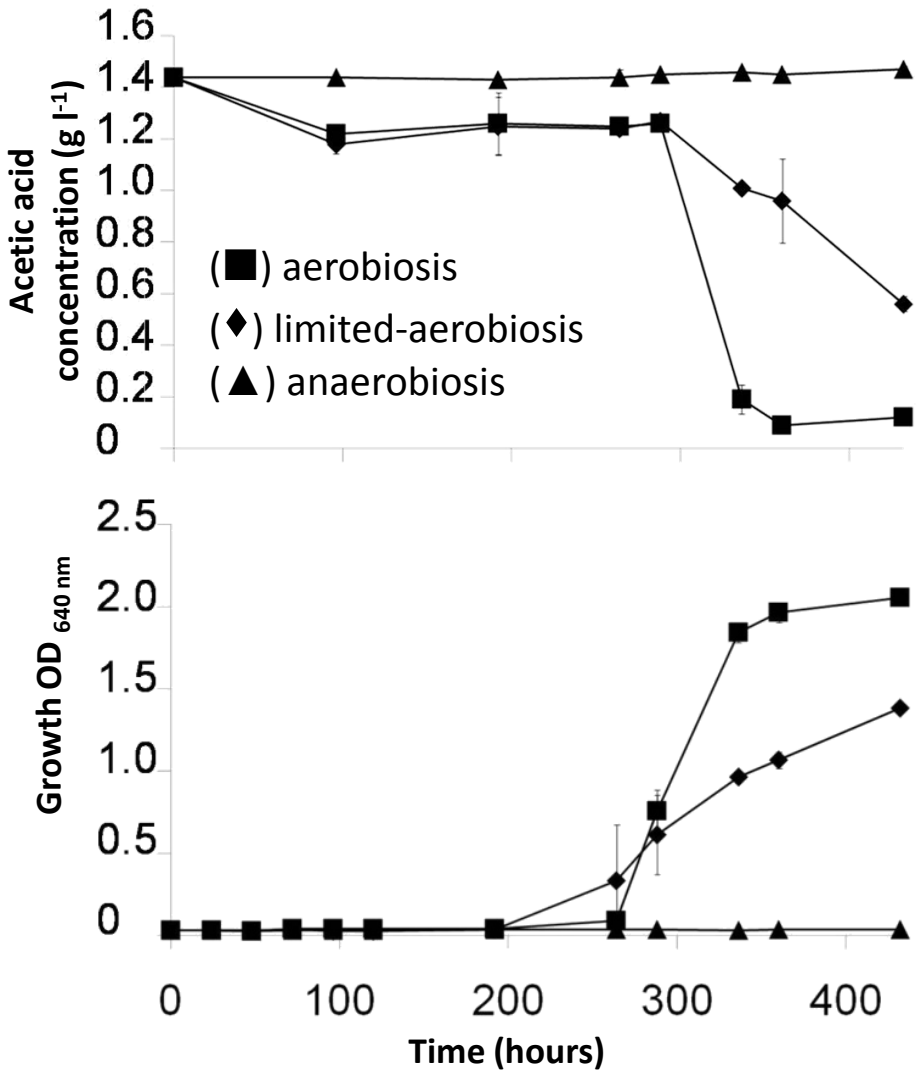
Development of a controlled process of volatile acidity (VA) bio-reduction in wines



Reduction of volatile acidity in simulated refermentation assays

	Acetic acid consumption (%)			
	Glucose (13%,w/v) and Ethanol (4%, v/v)		Glucose (3.3%,w/v) and Ethanol (10%, v/v)	
Yeast strains	Aerobic conditions	Limited-aerobic conditions	Aerobic conditions	Limited-aerobic conditions
***Z. bailii* ISA 1307**	94.8 ± 3.30	40.9 ± 9.80	71.2 ± 3.02	41.6 ± 2.64
***L. thermotolerans* 44C**	94.6 ± 4.79	15.25 ± 3.30	28.1 ± 1.70	17.4 ± 7.16
***S. cerevisiae* S26**	46.8 ± 4.99	45.9 ± 5.60	86.7 ± 2.63	44.6 ± 3.58
***S. cerevisiae* S29**	31.4 ± 2.47	82.5 ± 3.03	9.6 ± 3.03	43.3 ± 4.75

Reduction of volatile acidity of an acidic wine under different aeration conditions by S26 strain



Aeration conditions	Final VA (g l^{-1})	Acetic acid consumption (%)
Aerobiosis	0.12±0.04	89.6±2.97
Limited-aerobiosis	0.56±0.06	61.5±4.45
Anaerobiosis	1.47±0.00	0

Wine initial VA of 1.44 g l^{-1} and ethanol degree of 10.4 % (v/v)

Vilela-Moura *et al.*, 2008

Effect of acetic acid on cell viability and oenological parameters of an acidic wine by *S. cerevisiae* strains S26 and S29

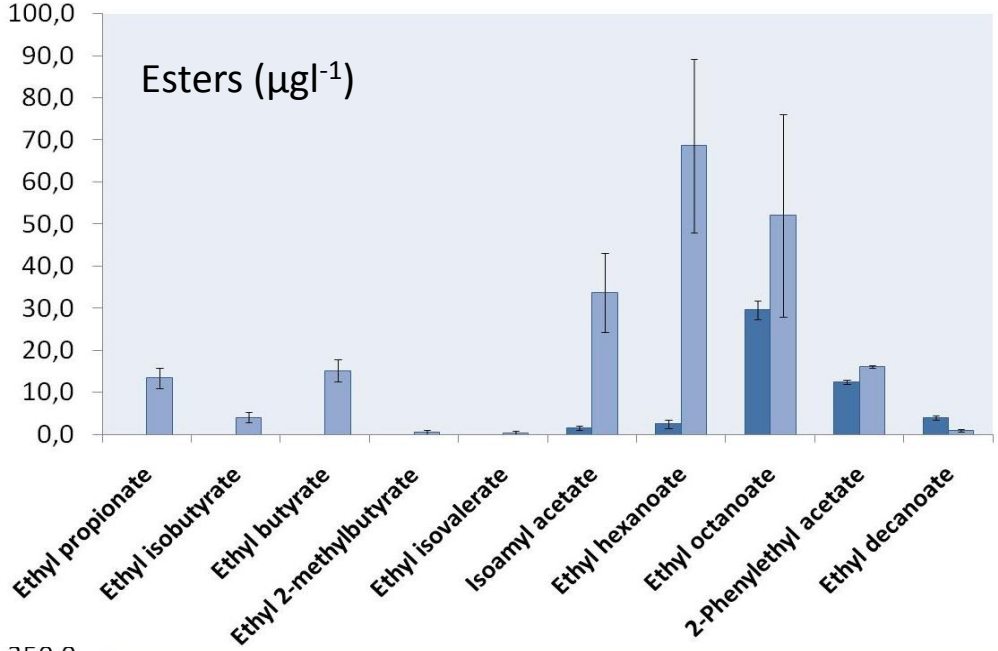
Strains	$[\text{Acetic acid}]_i$ (g l^{-1})	[Ethanol] % (v/v)	Acetic acid (% consump.)	CFU /ml (after 168 h.)
S26	1.0	10.3±0.1	78.0±2.65	10^7
S26	1.5	9.7±0.4	24.9±4.29	0
S26	1.75	9.8±0.2	21.7±0.99	0
S29	1.0	9.8±0.2	48.3±4.73	0
S29	1.5	9.7±0.2	8.9±3.08	0
S29	1.75	10.0±0.1	14.7±0.87	0

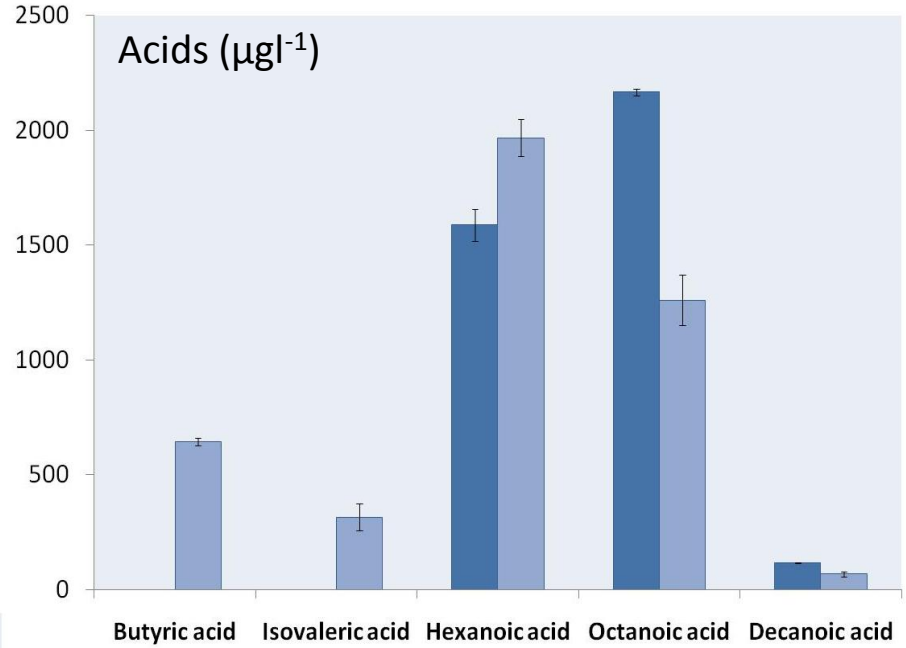
Wine initial ethanol (11.0 %, v/v), SO_2 (70 mg l^{-1}) and residual sugar (1.15 g l^{-1}), under limited-aerobic conditions

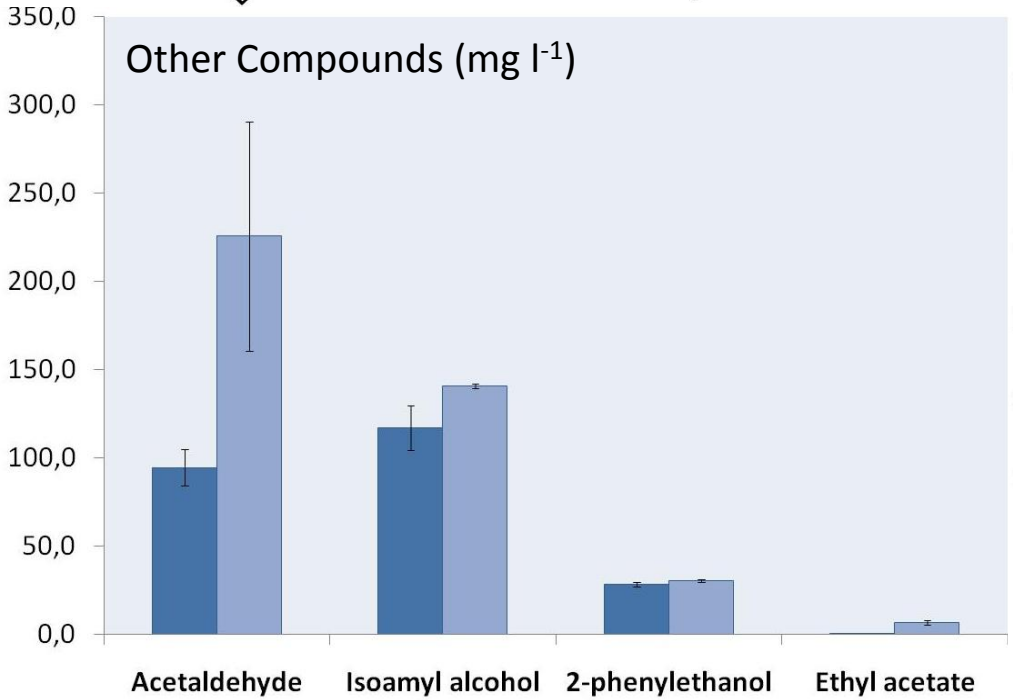
Vilela-Moura *et al.*, 2010a

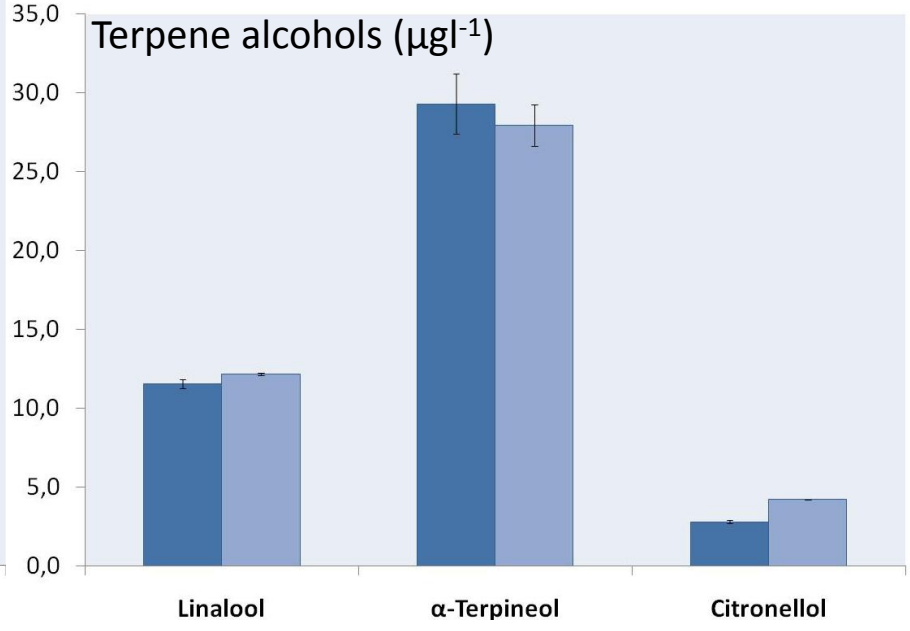
Aromatic composition of the deacidified wine

Wine prior deacidification
Wine after deacidification





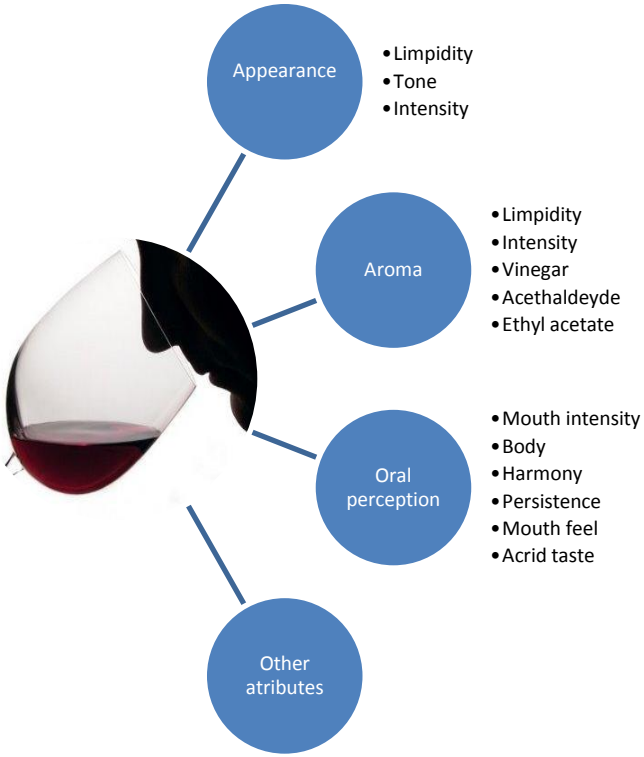


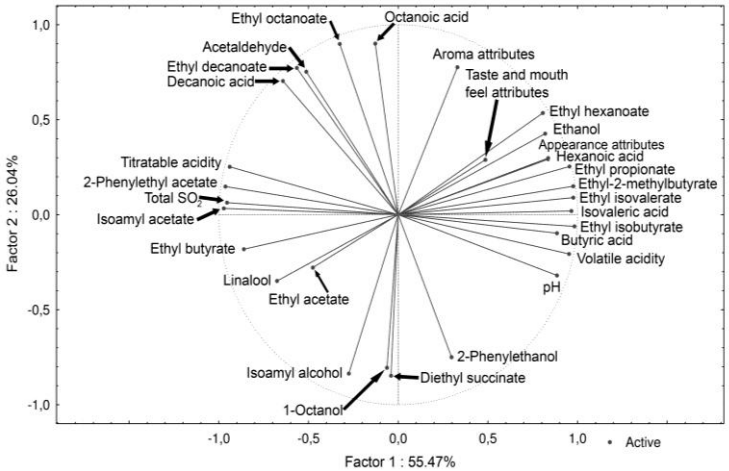


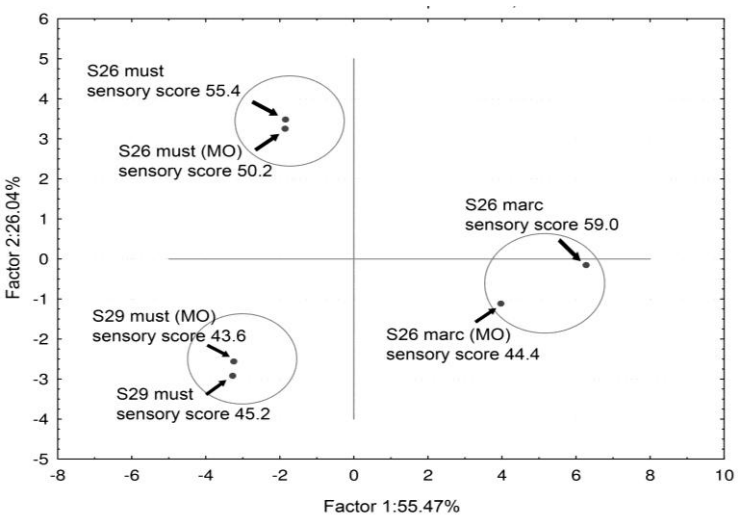
Acetic acid concentration and sensory score of acidic wines after refermentation with must or marcs

Conditions	Yeast strains	Acetic acid (g l^{-1})	Sensory score (total score)
Acidic white wine/must mixture		1.15	
Deacidified white wines	S26	0.61±0.02	n.d.
	S29	0.66±0.08	n.d.
	ISA 1307	0.83±0.02	n.d.
Acidic red wine/must mixture		1.12	
Deacidified red wines with MO	S26	0.42±0.06	50.2±5.5
	S29	0.38±0.11	43.6±9.3
Deacidified red wines without MO	S26	0.38±0.07	55.4±7.9
	S29	0.45±0.08	45.2±10.3
Acidic red wine/marc mixture		1.14	
Deacidified red wines with MO	S26	0.68±0.09	44.4±8.4
Deacidified red wines without MO	S26	0.69±0.05	59.0±7.2

PCA analysis
(chemical parameters and organoleptical evaluation)

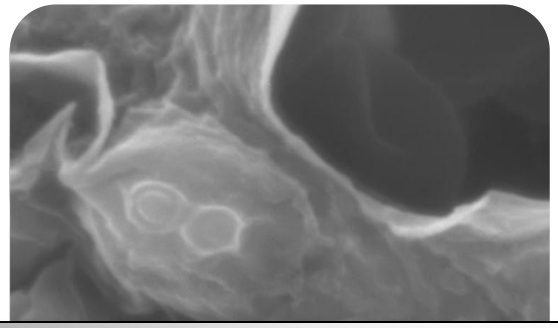


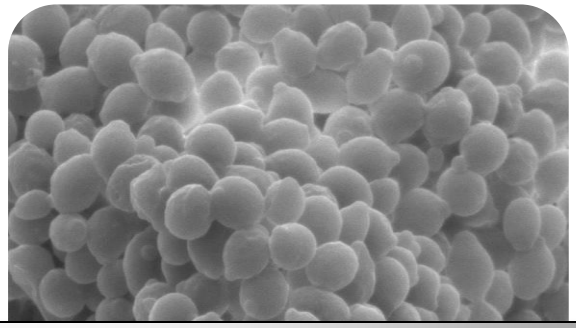




Vilela-Moura *et al.*, 2010b

Cells Immobilization

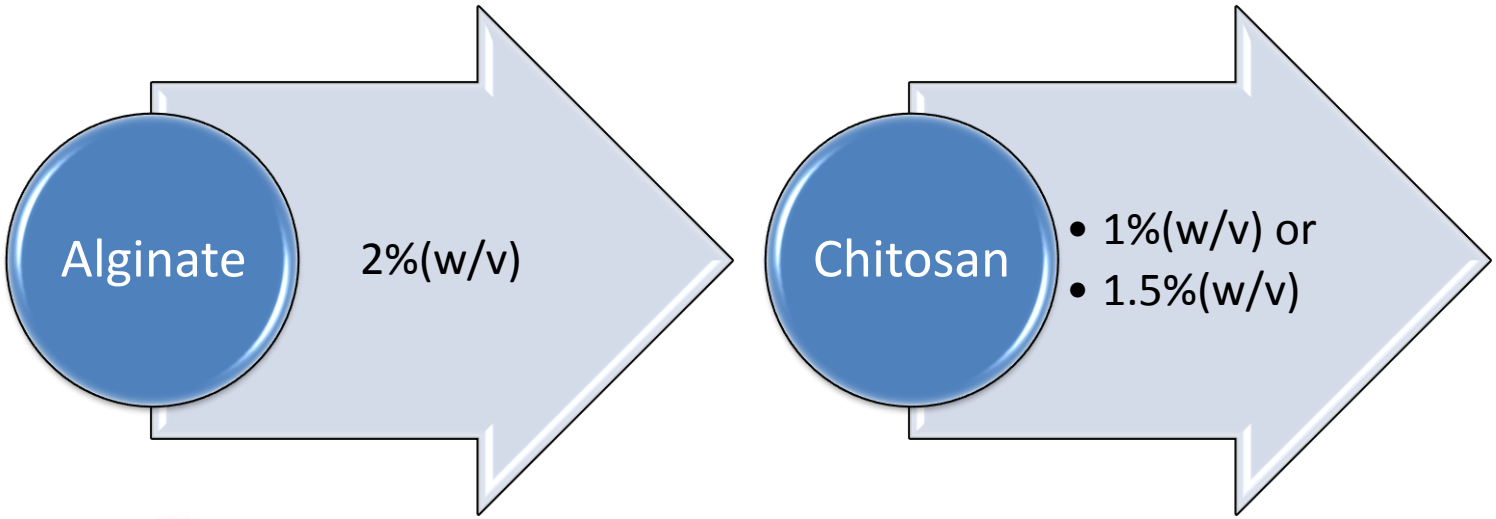




Reduction of volatile acidity of acidic wines by immobilized *Saccharomyces cerevisiae* cells

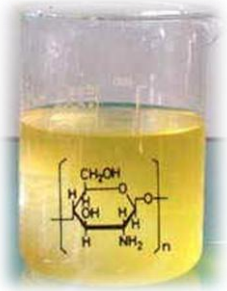
PATENTE DE INVENÇÃO NACIONAL n.º 105420 – Refª DMP/01/2012/335652 (submetido)

S26 *S. cerevisiae* cells immobilization process

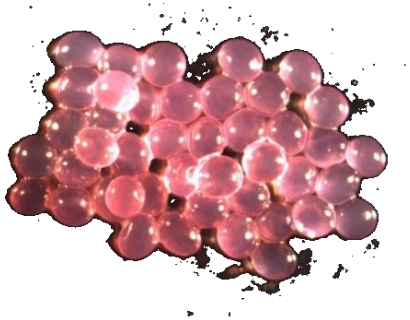


Alginate
and cells

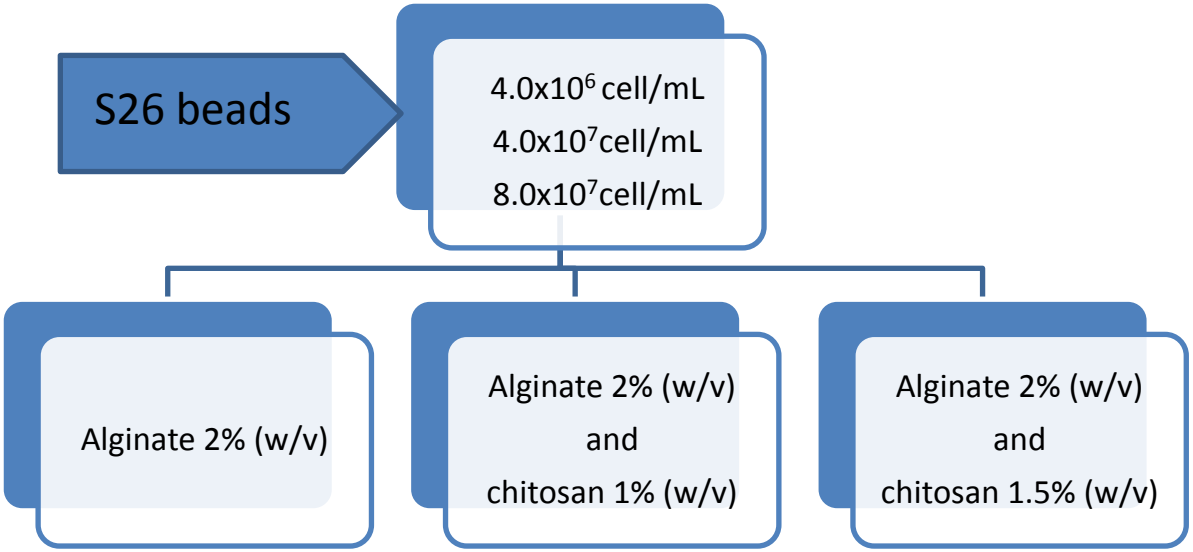
$CaCl_2$ (0.18 M)
1h at 4ºC



Alginate beads in chitosan
Over night, 100 rpm at 25 °C



S26 *S. cerevisiae* cells immobilization process



Glucose 200 gl^{-1}	Glucose 130 gl^{-1}	Glucose 50 gl^{-1}	Wine (1.10 $g.l^{-1}$ sugars), pH 3.5	Wine (1.10 $g.l^{-1}$ sugars), pH 3.12
- -	•Acetic acid 1.1 $g.l^{-1}$ •Ethanol 4%(v/v)	•Acetic acid 1.1 $g.l^{-1}$ •Ethanol 10%(v/v)	•Acetic acid 1.1 $g.l^{-1}$ •Ethanol 12.5%(v/v)	•Acetic acid (1.1 ; 1.3; 1.5 and 1.7 $g.l^{-1}$) •Ethanol 12.5%(v/v)

M.M. (van Uden, 1967)

Acetic acid consumption in minimal medium by immobilized cells

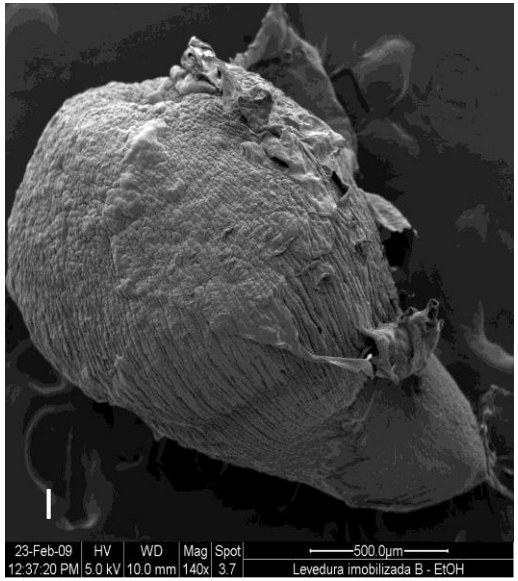
	One layer alginate beads				Double layer alginate-chitosan beads	
	$4.0x10^6$		$4.0x10^7$		$4.0x10^7$	
Media	48h	72h	48h	72h	48h	72h
	Glucose % **Acetic acid %** **CFU/ml**	**Glucose %** **Acetic acid %** **CFU/ml**	**Glucose %** **Acetic acid %** **CFU/ml**	**Glucose %** **Acetic acid %** **CFU/ml**	**Glucose %** **Acetic acid %** **CFU/ml**	**Glucose %** **Acetic acid %** **CFU/ml**
M.M. (A) Glucose 200 g l^{-1} pH 3.5			89.0±3.4 n.d. $3.3x10^7$		82.0±3.4 n.d. $3.0x10^4$	
M.M. (B) Glucose 130 g l^{-1} Acetic acid 1.1 g l^{-1} Ethanol 4% (v/v) pH 3.5	45.2±3.1 22.7±6.6 $3.5x10^6$		51.5±2.5 30.1±4.4 $1.5x10^7$		52.3±2.6 29.3±4.2 $2.6x10^4$	
M.M. (C) Glucose 50 g l^{-1} Acetic acid 1.1 g l^{-1} Ethanol 10% (v/v) pH 3.5		32.4±0.3 23.0±7.6 $4.0x10^4$		51.9±9.1 33.8±5.3 $5.5x10^7$		53.4±8.6 31.4±7.8 $5.5x10^4$

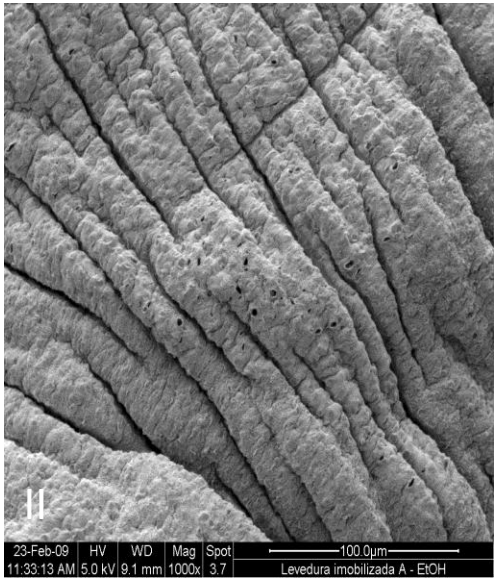
Acetic acid consumption in wine by immobilized cells

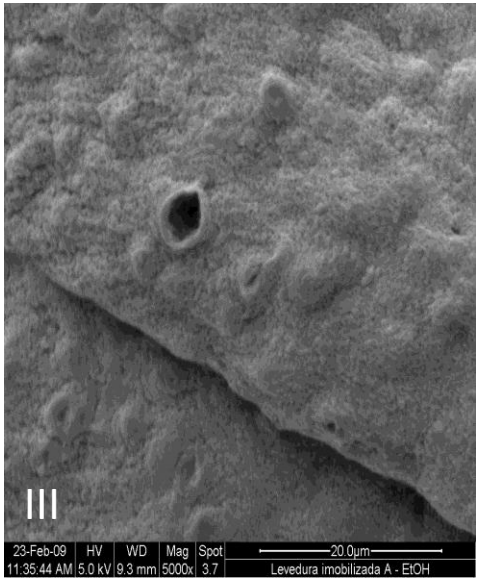
	One layer alginate beads		Double layer alginate-chitosan beads			
	$4.0x10^7$		$4.0x10^7$		$8.0x10^7$	
Wine	48h	72h	48h	72h	48h	72h
	Acetic acid % **CFU/ml**	Acetic acid % **CFU/ml**	Acetic acid % **CFU/ml**	Acetic acid % **CFU/ml**	Acetic acid % **CFU/ml**	Acetic acid % **CFU/ml**
Wine (D) Acetic acid 1.1 g l^{-1} pH 3.5	23.5±5.2 $3.3x10^6$	28.6±3.2 $2.0x10^7$	17.9±1.2 $4.4x10^3$	21.6±2.3 $3.5x10^4$	25.6±1.6 $5.6x10^3$	28.1±3.2 $6.7x10^4$
Wine (E) Acetic acid 1.1 g l^{-1} pH 3.12	23.1±5.7 0.0±0.0	24.9±5.2 0.0±0.0	24.8±9.7 0.0±0.0	27.3±5.1 0.0±0.0	31.1±3.4 0.0±0.0	42.9±2.4 0.0±0.0
Wine (F) Acetic acid 1.3 g l^{-1} pH 3.12					26.4±2.7 0.0±0.0	34.0±3.3 0.0±0.0
Wine (G) Acetic acid 1.5 g l^{-1} pH 3.12					25.9±0.1 0.0±0.0	32.7±4.4 0.0±0.0
Wine (H) Acetic acid 1.7 g l^{-1} pH 3.12					21.7±0.3 0.0±0.0	26.0±0.8 0.0±0.0

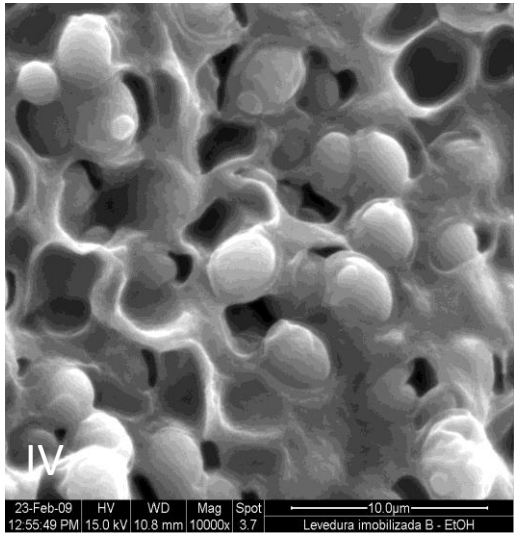
Wine residual sugar of 1.12 g l^{-1} and ethanol 12.5% (v/v)

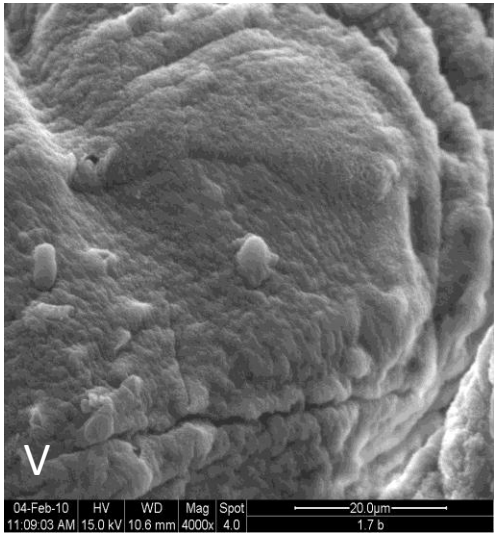
Scanning electron microscopy (SEM) analysis of immobilised cells

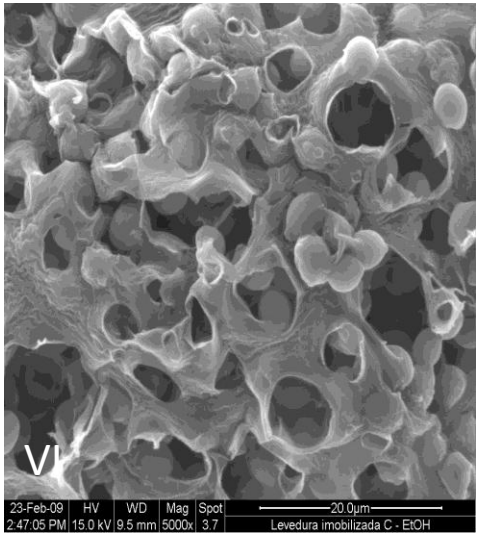












Concluding remarks

- *S. cerevisiae* S26 cells entrapped in double-layer alginate-chitosan beads efficiently remove the acetic acid from an acidic wine (1.1 g l^{-1}) acetic acid, 12.5% ethanol, pH 3.12)

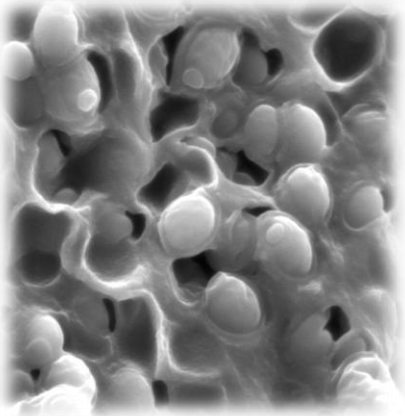
- Immobilized S26 cells display higher ethanol tolerance, when compared with cells in suspension

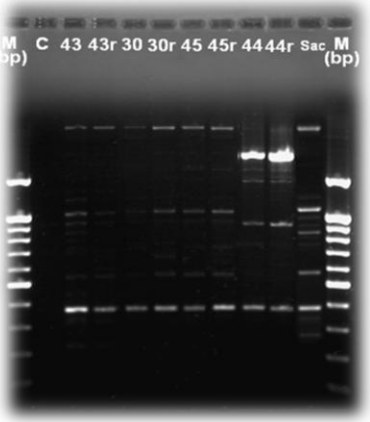
- Immobilized S26 cells may be applied to correct excessive volatile acidity and therefore improve the global quality of wines in a cheap, simple and efficient way

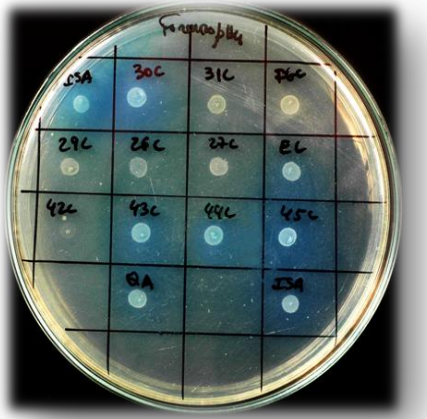
Future directions

- Scale up the immobilization process and study the applicability of immobilized *S. cerevisiae* cells to different types of wines with high levels of volatile acidity.

- Organoleptical evaluation of the final deacidified wines.




M (bp)
C 43 43r 30 30r 45 45r 44 44r Sac
M (bp)


29A
30C
31C
26C
29C
26C
27C
EC
42C
43C
44C
45C
QA
29A