INSTITUTE FOR BIOTECHNOLOGY AND BIOENGINEERING

Centre of Genetics and Biotechnology of the University of Trás-os-Montes and Alto Douro

Centro de Biologia Molecular e Ambiental
Universidade do Minho

Vilela-Moura A.
Schuller D.
Mendes-Faia A.
Côrte -Real M.

THE EFFECT OF MICRO-OXYGENATION AND CELL IMMOBILIZATION ON THE REDUCTION OF EXCESSIVE VOLATILE ACIDITY FROM WINES

An enological problem

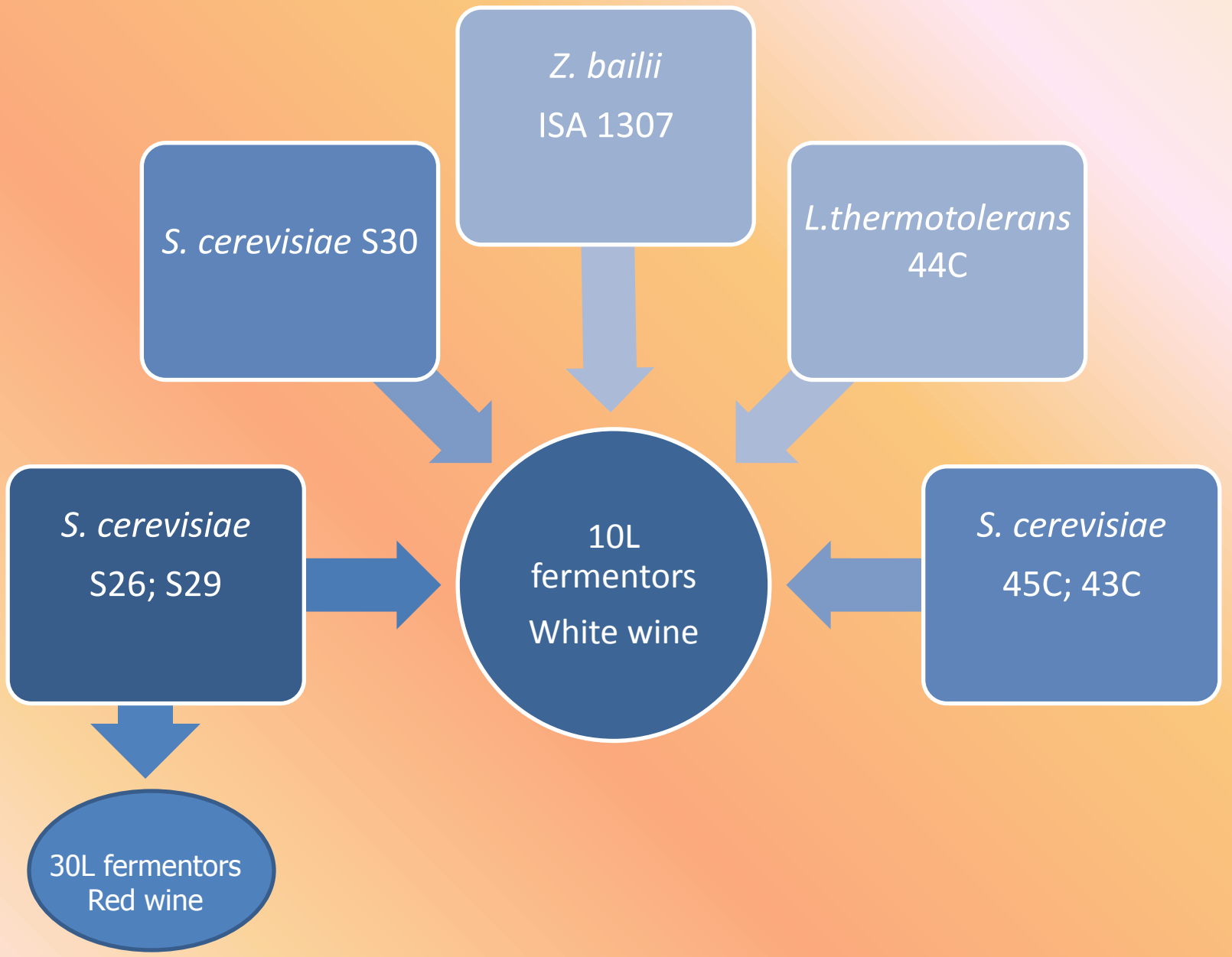
- **Acetic acid is the main component of volatile acidity, and critical for wine quality;**
- **This acid is mainly produced by bacterial spoilage and *Botrytis cinerea* infecting grapes; also formed by yeasts during alcoholic fermentation;**
- **Above 0.8 $g.l^{-1}$, acetic acid has a detrimental organoleptical effect (acidic wine).**

Aims of the study

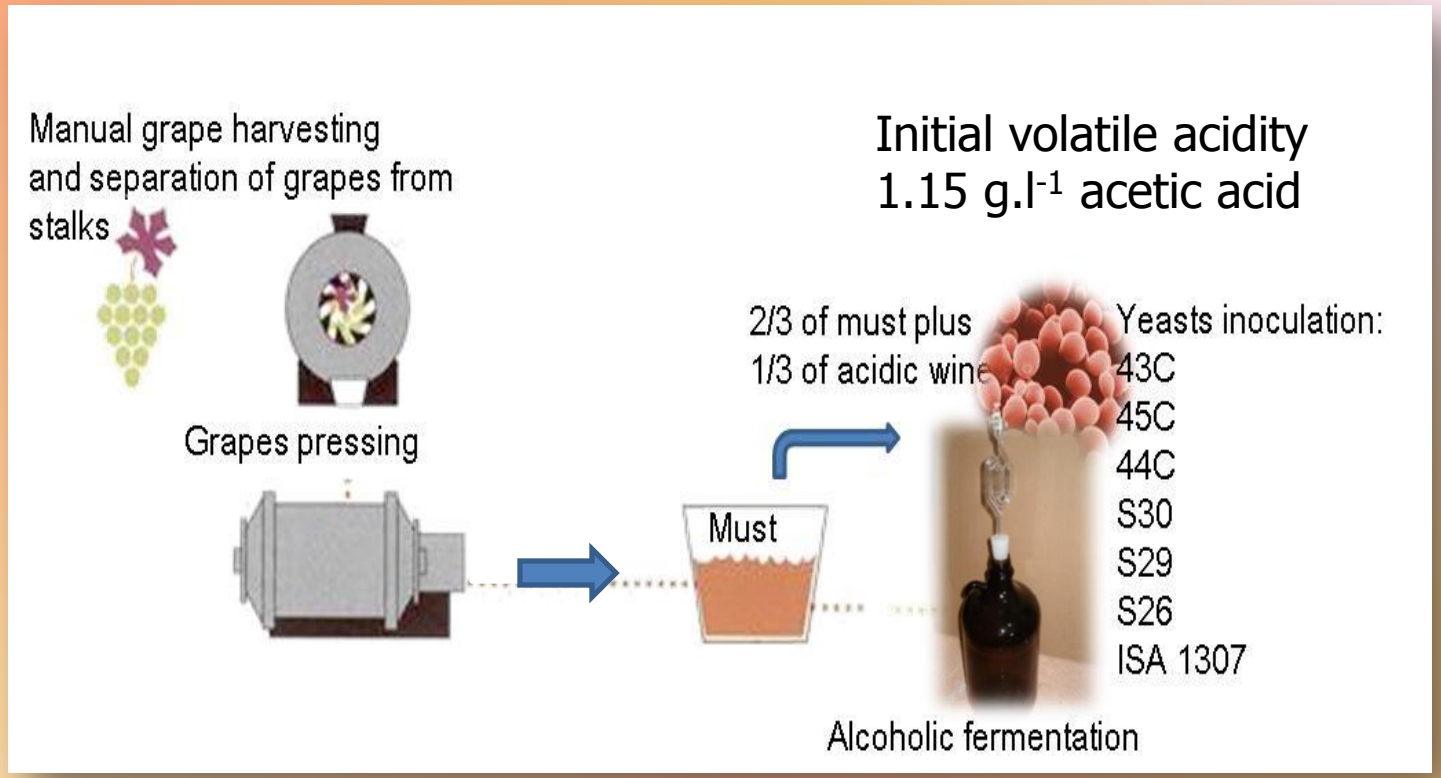
To evaluate:

- **the decrease of volatile acidity from acidic wines by *S. cerevisiae* strains**
- **the effect of micro-oxygenation on wine deacidification and aroma composition**
- **the efficiency of removal acetic acid by immobilized *S. cerevisiae* S26**

yeast strains tested in “remostagem” assays:



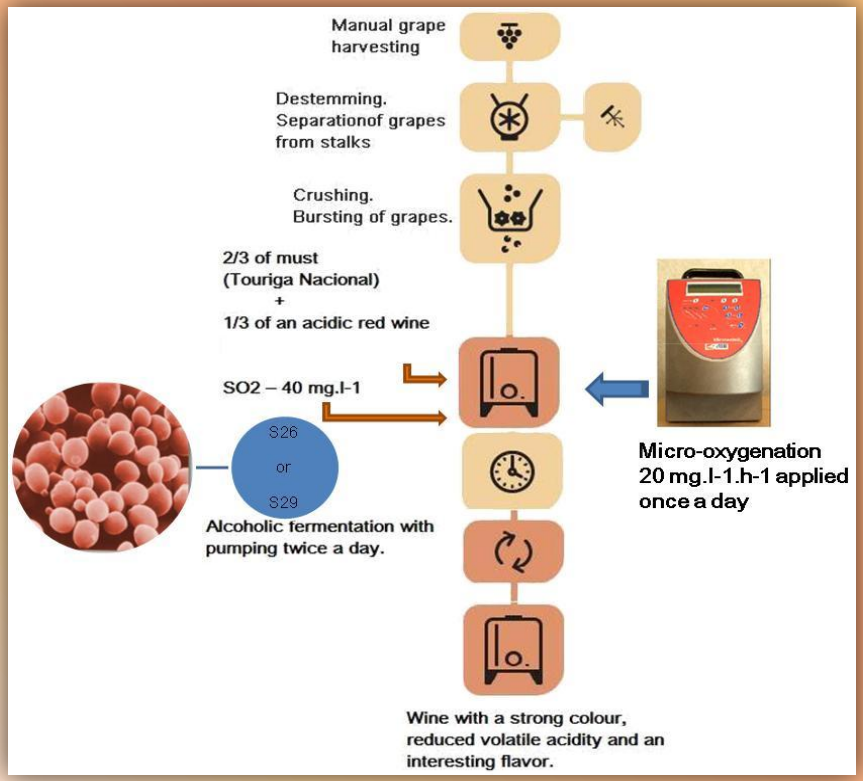
“remostagem” process: white wine



“remostagem” process: red wine

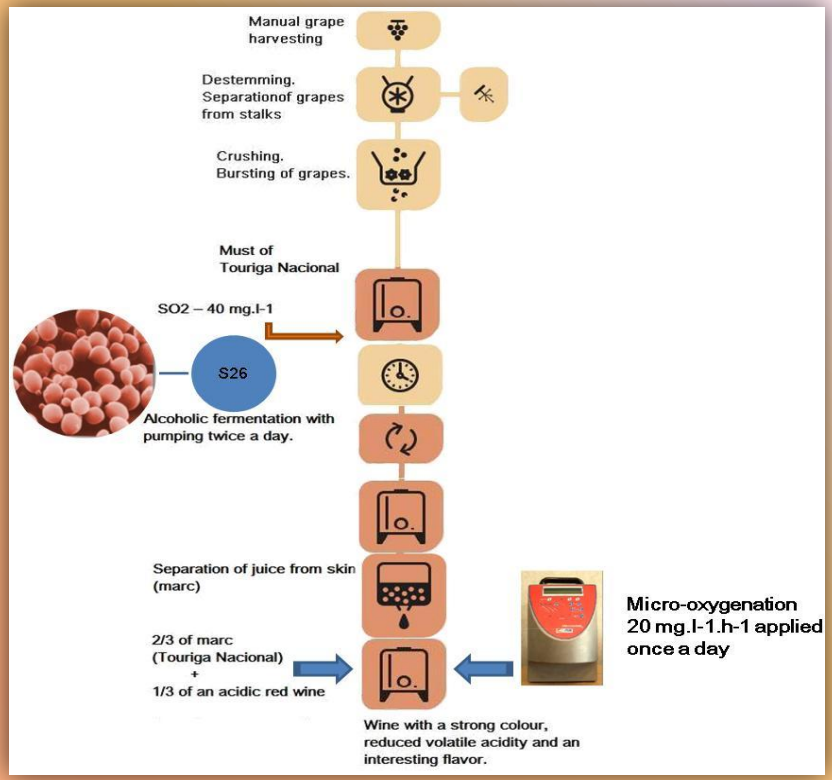
Musts

Initial volatile acidity
1.12 g.l^{-1} acetic acid



Marcs

Initial volatile acidity
1.14 g.l^{-1} acetic acid



Acetic acid and glucose consumption by seven strains tested in grape must (Viosinho)

White wine refermentation with must of Viosinho variety

(Limited-aerobic conditions)

Yeast strains	48 hours of refermentation **Acetic acid (%) consumption** Glucose (%) consumption	264 hours of refermentation **Acetic acid (%) consumption** Glucose (%) consumption
43C	**29.6 ± 2.61** [a]	**37.4 ± 6.90** [a]
	0.0 ± 0.0 [a]	99.5 ± 0.83 [a]
44C	**20.6 ± 0.50** [b]	**(Increased) 10.4 ± 3.01** [c]
	0.0 ± 0.0 [a]	35.1 ± 1.36 [b]
45C	**28.1 ± 1.33** [a, c]	**41.7 ± 1.51** [a, b]
	0.0 ± 0.0 [a]	100.0 ± 0.0 [a]
S26	**30.7 ± 1.33** [a]	**47.0 ± 1.51** [b]
	18.0 ± 1.11 [c]	100.0 ± 0.0 [a]
S29	**23.3 ± 1.33** [b, c]	**42.6 ± 1.33** [a, b]
	16.8 ± 1.15 [b]	100.0 ± 1.0 [a]
S30	**28.7 ± 1.51** [a]	**36.5 ± 1.51** [a]
	0.0 ± 0.0 [a]	98.0 ± 0.66 [a]
ISA 1307	**28.7 ± 1.51** [a]	**27.8 ± 0.00** [d]
	0.0± 0.0 [a]	79.0 ± 1.60 [b]

Acetic acid and glucose consumption by two strains tested in red grape must or in marc

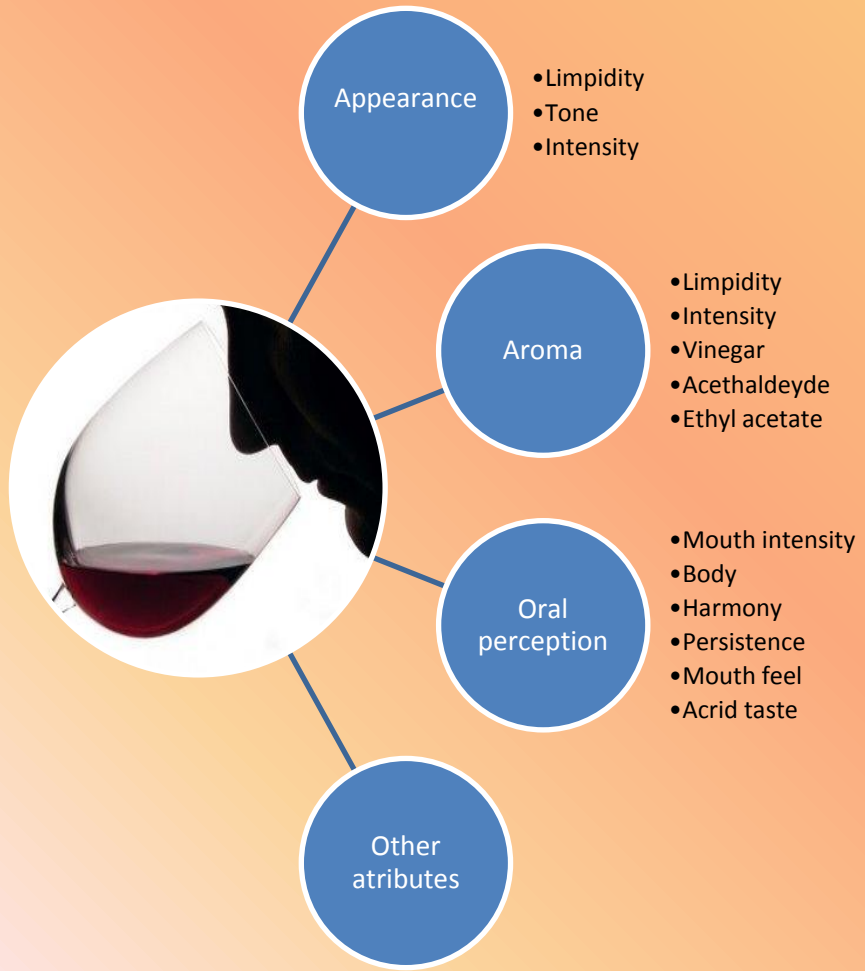
	Red wine refermentation with must				Red wine refermentation with marc			
	48 h		264 h		72 h		96 h	
	Limited-aerobic conditions plus one hour/day micro-oxygenation	Limited-aerobic conditions	Limited-aerobic conditions plus one hour/day micro-oxygenation	Limited-aerobic conditions	Limited-aerobic conditions plus one hour/day micro-oxygenation	Limited-aerobic conditions	Limited-aerobic conditions plus one hour/day micro-oxygenation	Limited-aerobic conditions
Yeast strains	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose	**Acetic acid** Glucose
S26	**35.7 ± 1.57** [a] 20.6 ± 4.33 [a]	**37.5 ± 4.72** [a] 14.6 ± 6.51 [a]	**66.1 ± 6.30** [b] 100.0 ± 0.0 [b]	**62.5 ± 5.45** [b] 100.0 ± 0.0 [b]	**38.6 ± 3.15** [a] 100.0 ± 0.0 [b]	**41.2 ± 6.86** [a] 100.0 ± 0.0 [b]	**40.4 ± 4.17** [a] 100.0 ± 0.0 [b]	**39.5 ± 8.18** [a] 100.0 ± 0.0 [b]
S29	**42.9 ± 5.68** [a] 25.8 ± 10.10 [a]	**44.6 ± 12.30** [a] 23.8 ± 4.10 [a]	**59.8 ± 7.22** [b] 100.0 ± 0.0 [b]	**66.1 ± 9.58** [b] 100.0 ± 0.0 [b]	-	-	-	-

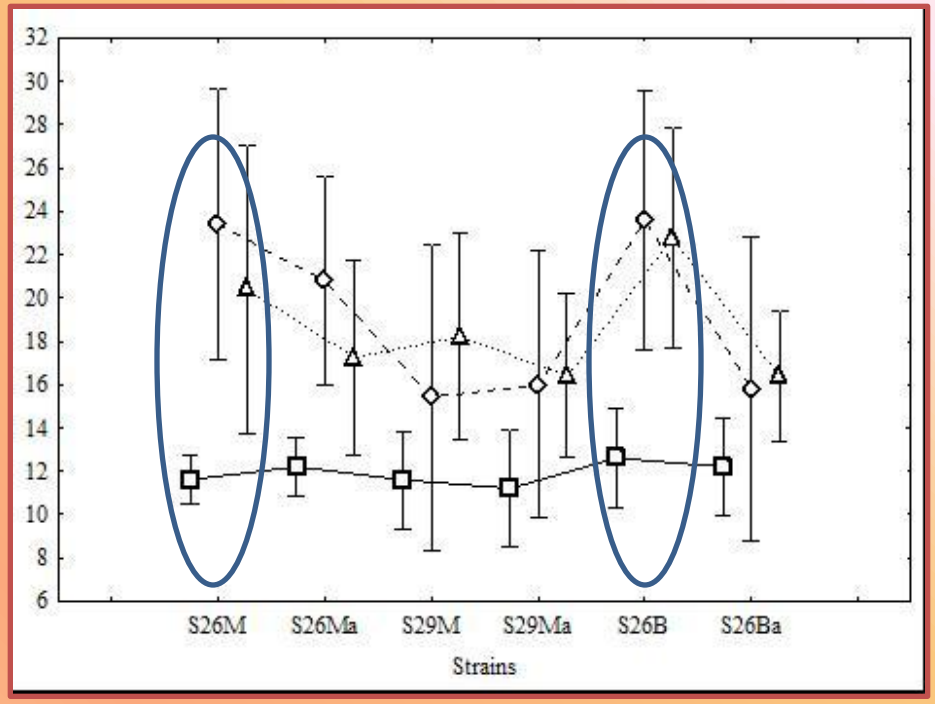
Characterisation of the wines obtained after deacidification processes

Conditions	Strains	Ethanol % (v/v)	pH	Acetic acid ($g.l^{-1}$)	Titratable acidity ($g.l^{-1}$)	Total SO_2 ($mg.l^{-1}$)	Free SO_2 ($mg.l^{-1}$)
White wine							
Limited-aerobic	43C must	11.9±0.14	3.16±0.01	0.72±0.08	7.13±0.95	36.1±3.61	0.80±0.68
	S26 must	12.1±0.04	3.19±0.01	0.61±0.02	6.62±0.19	33.3±1.08	0.48±0.23
	45C must	11.9±0.11	3.14±0.01	0.67±0.02	6.73±0.24	39.4±2.54	0.91±0.98
	S30 must	11.8±0.04	3.16±0.01	0.73±0.02	7.11±0.18	37.9±3.26	0.64±0.45
	44C must	8.0±5.59	3.08±0.03	1.40±0.20	8.63±1.59	4.6±3.53	1.14±1.56
	ISA 1307 must	11.4±0.11	3.17±0.01	0.83±0.02	6.75±0.08	38.4±3.26	0.32±0.98
	S29 must	11.9±0.04	3.19±0.01	0.66±0.08	6.62±0.04	34.3±2.54	0.33±0.23
Red wine							
Limited-aerobic	S26 must	11.8±0.2	3.29±0.02	0.42±0.06	8.35±0.61	115.01±4.86	0.0±0.0
	S29 must	11.3±0.1	3.31±0.03	0.38±0.11	7.80±0.27	130.71±17.05	0.0±0.0
	S26 marc	12.1±0.6	3.44±0.03	0.69±0.09	7.46±0.16	46.93±2.96	0.0±0.0
Micro-oxyg	S26 must	11.1±0.3	3.29±0.05	0.38±0.07	8.35±0.17	105.22±15.57	0.0±0.0
	S29 must	11.0±0.3	3.32±0.05	0.45±0.08	8.03±0.40	102.57±2.24	0.0±0.0
	S26 marc	11.9±0.3	3.50±0.03	0.68±0.05	7.56±0.49	56.32±7.13	0.0±0.0

Organoleptical evaluation

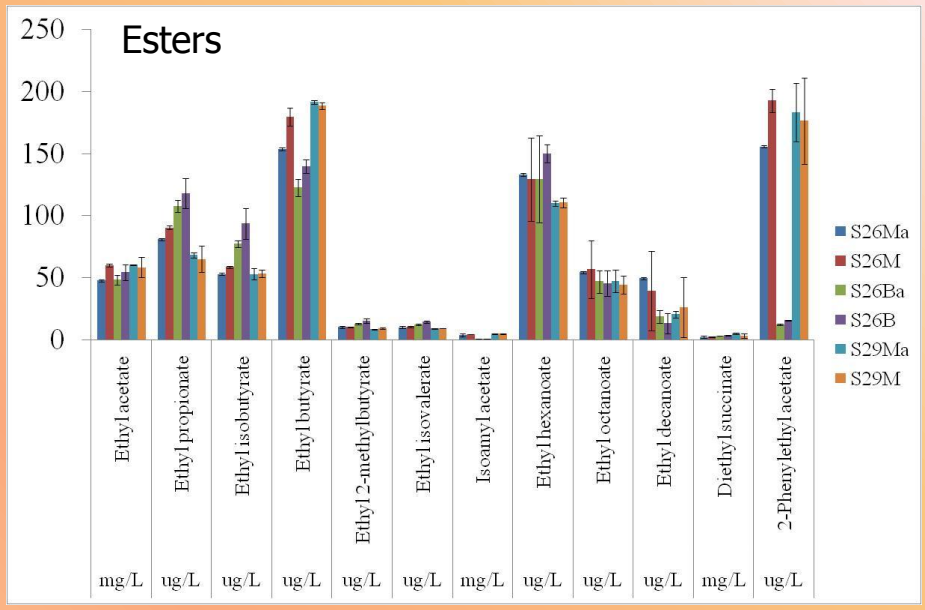
- Trained panel of 5 judges
- The attributes were quantified using a six-point intensity scale

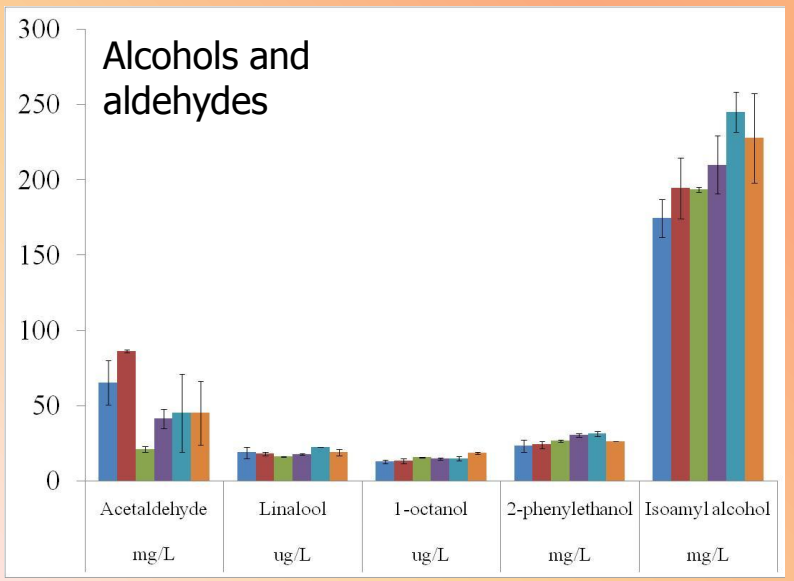


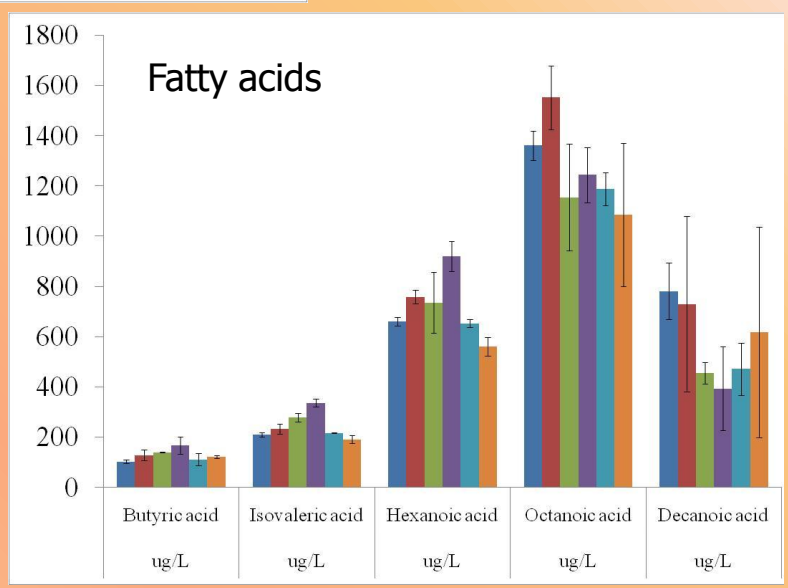


Average scores for appearance (□), aroma (◊) and taste (△) attributes

SPME - GC-MS Analysis of the wines



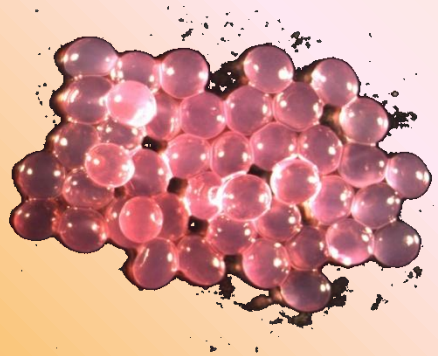




Ongoing work : S26 cell immobilization in double layer alginate-chitosan beads

Alginate → 2%(w/v)

Chitosan → • 1%(w/v) or • 1.5%(w/v)



Alginate and cells

$CaCl_2$ (0.18 M)
1h at 4 ºC

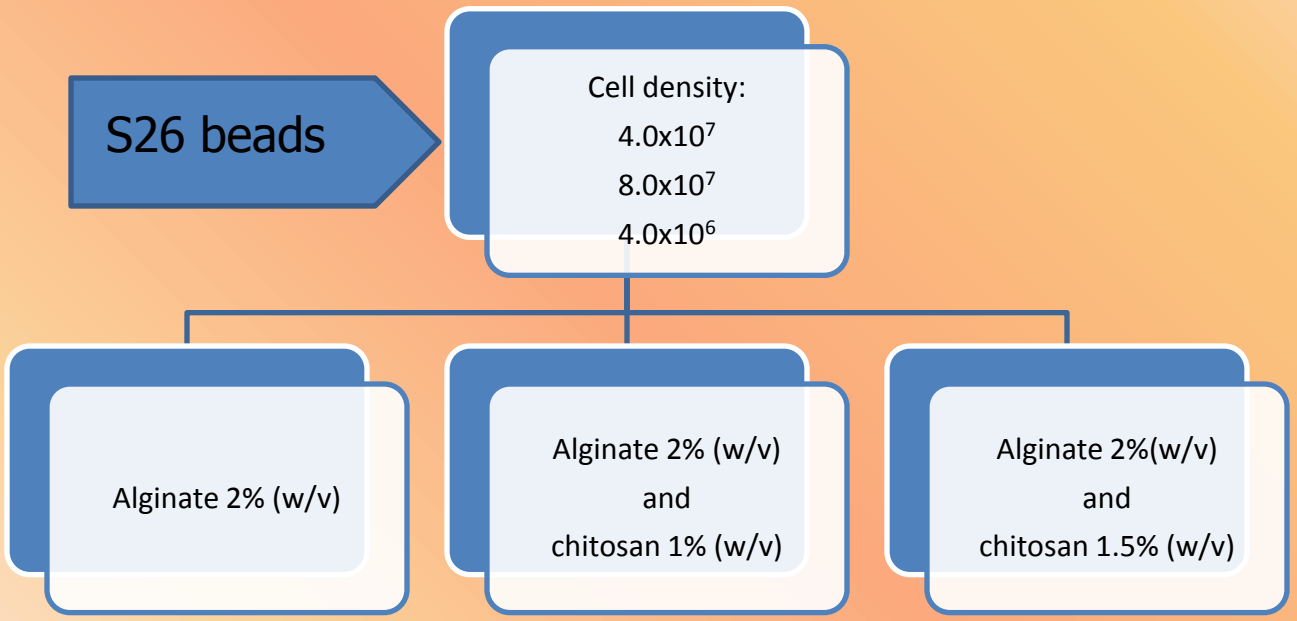
Alginate beads in chitosan
0vernight, 100 rpm
at 25 ºC

Double layer alginate-chitosan beads:

deacidification assays

Glucose 200 $g.l^{-1}$	Glucose 130 $g.l^{-1}$	Glucose 50 $g.l^{-1}$	Wine (1.10 $g.l^{-1}$ sugars)
• -- • --	• 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)

Mineral medium (van Uden, 1967)

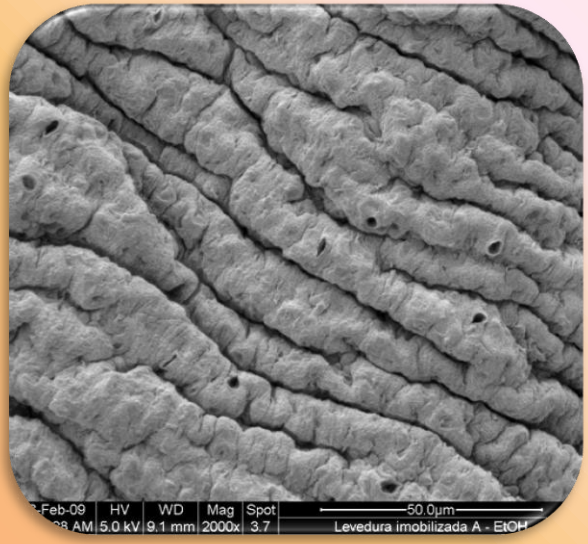


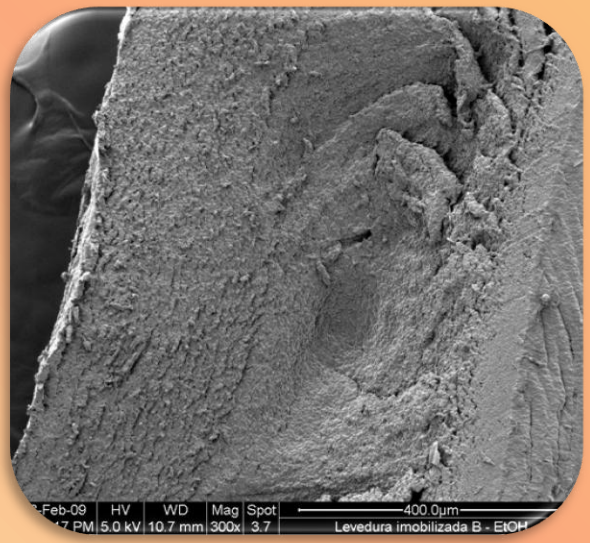
Acetic acid consumption and cell leakage after refermentation assays with entrapped S26 cells

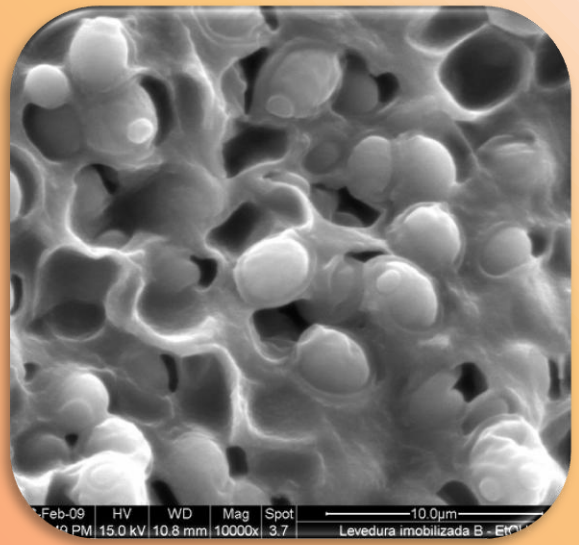
	Alginate 2% (w/v)				Alginate 2% (w/v) and chitosan 1% (w/v)			
	$4.0x10^6$		$4.0x10^7$		$4.0x10^7$		$8.0x10^7$	
Media	48h	72h	48h	72h	48h	72h	48h	72h
	Acetic acid% Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}	**Acetic acid%** Cell ml^{-1}
Glucose 130g.l^{-1} Acetic acid 1.1 g.l^{-1} Ethanol 4%(v/v)	**21.0 ± 6.6** ($35x10^5$)	-	**29.1 ± 4.4** ($15x10^6$)	-	**30.2 ±4.2** ($26x10^3$)	-	-	-
Glucose 50 g.l^{-1} Acetic acid 1.1 g.l^{-1} Ethanol 10%(v/v)	-	**21.9 ±7.6** ($40x10^3$)		**34.5 ± 5.3** ($55x10^6$)	-	**34.4 ± 7.8** ($55x10^3$)*	-	-
Wine (1.10 g.l^{-1} sugars) Acetic acid 1.1 g.l^{-1} Ethanol 12.5%(v/v)	-	-	**24.5 ± 5.2** ($35x10^5$)	**29.6 ± 3.2** ($20x10^6$)	**18.7 ± 1.2** ($44x10^2$)*	**22.1 ± 2.3** ($35x10^3$)*	**26.4 ± 1.55** ($56x10^2$)*	**29.1 ± 3.2** ($67x10^3$)*

* Cell flocculation

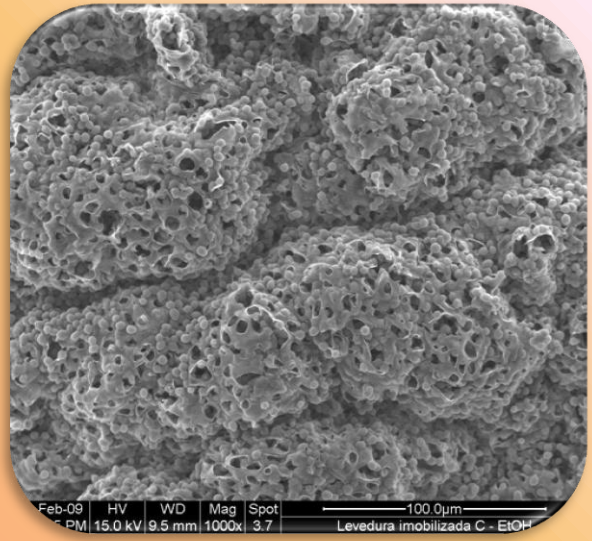
SEM images: beads prior use

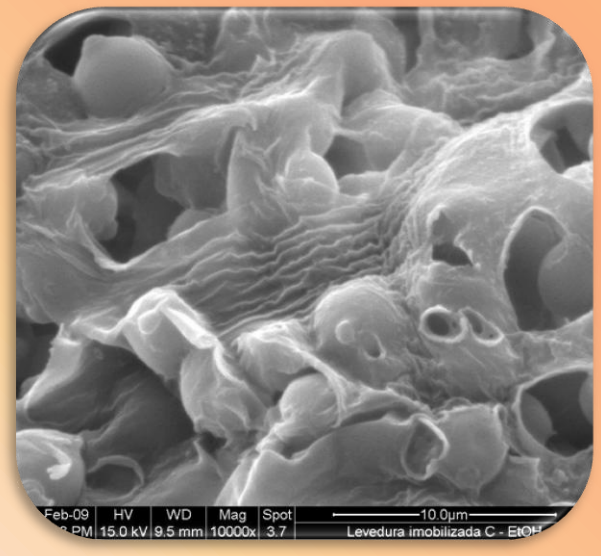


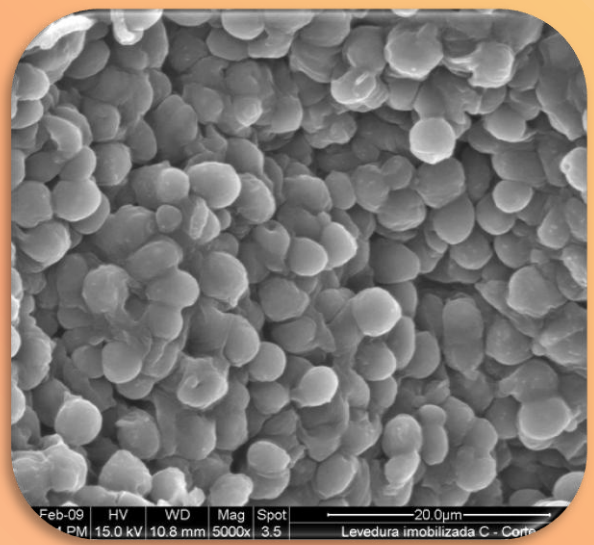




SEM images: beads after wine deacidification







Final Remarks

- Generally, the *S. cerevisiae* strains characterized herein are capable to remove acetic acid from acidic white or red wines during a refermentation process

  - *S. cerevisiae* strain S26 is the most efficient acid degrading strain in both refermentation processes but its efficiency is higher in red acidic wines;
  - Acetic acid removal efficiency was obtained for initial concentrations about two-fold higher (1.1 g l^{-1}) than the values proposed for a typical refermentation assay (0.6 g.l^{-1});
  - Micro-oxygenation was not a key factor for acetic acid removal;
  - The refermented wines treated with micro-oxygenation revealed a vegetable character and mouth hardness in comparison to the more floral notes that predominated in wines obtained without micro-oxygenation;
  - Immobilized cells of S26 strain can decrease volatile acidity of wines with ethanol up to 12.5% and 1.1 g l^{-1} of acetic acid;
  - Cell leakage is lower in beads with alginate-chitosan double layer beads.

Future perspectives

- Evaluate the capacity of entrapped cells of *S. cerevisiae* S26 and S29 to perform biological deacidification of wines with excessive acetic acid either directly or through a “remostagem” process at an industrial scale;

- Evaluate fermentative profiles and sensory properties of wines deacidified by *Saccharomyces cerevisiae* entrapped cells.

Acknowledgements

Universidade de Trás-os-Montes e Alto Douro

Arlete Faia
Virgílio Falco
Pedro Tavares

Universidade do Minho

Manuela Côrte-Real
Dorit Schuller