

# 3<sup>rd</sup> European Food Safety and Standards Conference

October 24-25, 2016 Valencia, Spain

POLITÉCNICA DE MADRID

[www.upm.es](http://www.upm.es)

October, 2016

Non-*Saccharomyces* yeasts *Lachancea thermotolerans* and *Schizosaccharomyces pombe* mixed cultures applications in wine food safety (biogenic amines and ethyl carbamate control) from high pH grape juice

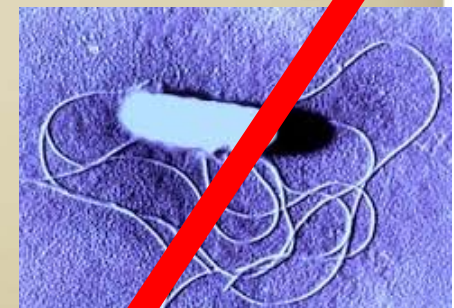
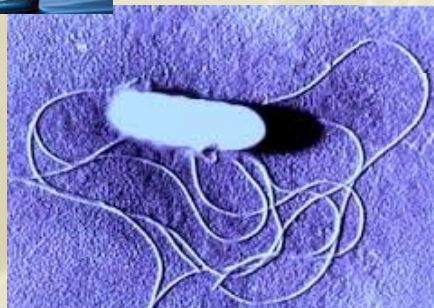
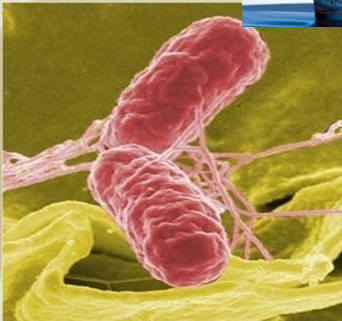
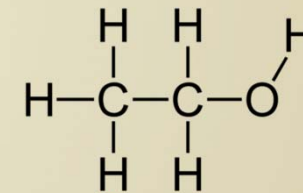
Dept. Chemistry and Food Technology  
Prof. Santiago Benito Sáez.

# Lecture objectives

- To give a summary about red wine and Food Safety => Main problems ↔ possible industrial solutions.
- To propose a specific alternative in order to manage two specific wine /Food Safety emerging problems:
  - Biogenic amines.
  - Ethyl Carbamate.
- To propose an alternative to increase wine quality in high ph grape juice.

# Introduction

Wine => Easy to manage from Food Safety point of view.

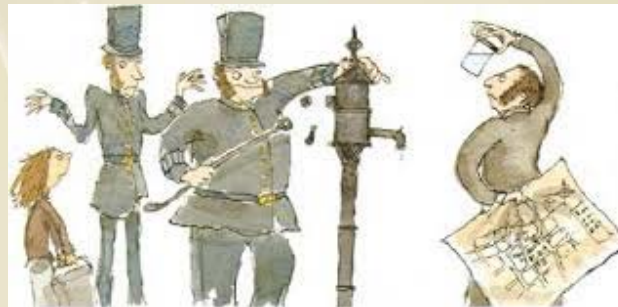


# Introduction

London 1854 Broad Street (Cholera outbreak)  
What would you prefer to drink ?



**NOTICE.**  
**PREVENTIVES OF**  
**CHOLERA!**  
Published by order of the Sanatory Committee, under the sanction of the Medical Council.  
**BE TEMPERATE IN EATING & DRINKING!**  
*Avoid Raw Vegetables and Unripe Fruit !.*  
Abstain from **COLD WATER**, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.



# Today everything is different

There are several problems related to wine - food safety  
(No high risk microorganisms => but other significant risks)



Alcoholism

Ochratoxin  
A

Physical  
hazards



SO<sub>2</sub> or other  
preservatives



Aditives  
(Clarification)



Biogenic  
Amines

Ethyl  
Carbamate



# Summary

## Main Wine Food Safety problems <=> Solutions

<b>Problem</b>	<b>Risk group</b>	<b>Solution (HACCP)</b>
Ethanol	Alcoholics, diabetics,	

# General problems related to alcohol-alcoholism



**Most alcoholics do not drink wine (Spanish Society of Anonymous Alcoholics)**

# General problems related to alcohol



**Most alcoholics do not drink wine (Spanish Society of Anonymous Alcoholics)**



**Most wine consumers=> Considered as responsible => Things ≠Alcohol**



# General problems related to alcohol



**Most alcoholics do not drink wine (Spanish Society of Anonymous Alcoholics)**



**Most traffic accidents related to alcohol consumption do not depend on wine (Spanish Traffic Agency)**

Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics.....	Labeled Free alcohol wine



Responsible Consumers

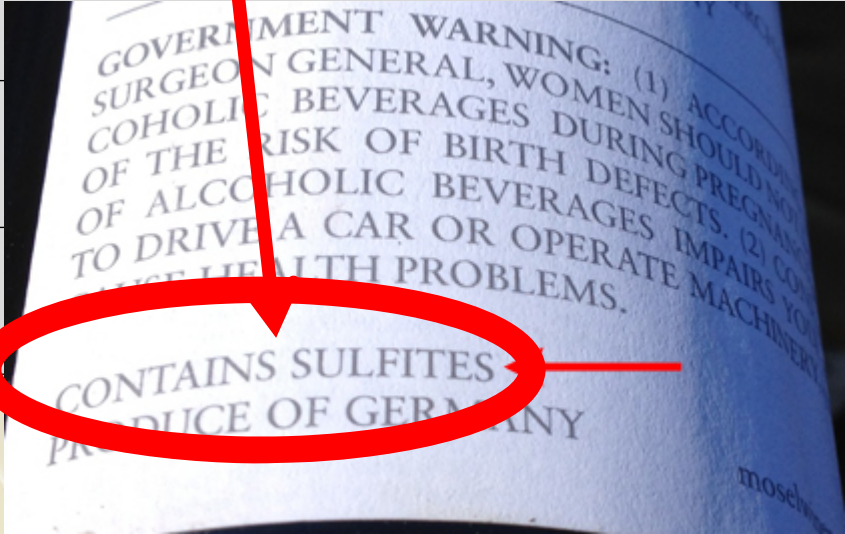
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Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	

Problem	Risk group	Solution (HACCP)
Physical hazards	Anyone	Filtration



<b>Problem</b>	<b>Risk group</b>	<b>Solution (HACCP)</b>
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SO <sub>2</sub>	Asthmatics	

Problem	Risk group	Solution (HACCP)
SO <sub>2</sub>	Asthmatics	<p>Labeled: It contains sulfites.</p> <p>Other preservatives (sulfites free)</p> <p>Legal limits</p>



Use other new preservatives or technologies

<b>Problem</b>	<b>Risk group</b>	<b>Solution (HACCP)</b>
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
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Allergenic additives	allergic people (Ex: albumin)	

Problem	Risk group	Solution (HACCP)
Allergenic additives	allergic people (Ex: albumin)	<p style="color: red; text-align: center;">Labeled.</p> <p style="color: red; text-align: center;">Or non allergenic additives</p>

**IS YOUR WINE VEGAN?**  
fining agents commonly used during the filtering process

**albumin**



The white or not clear part surrounding the yolk. Also called the egg white.

**isinglass**



substance obtained from dried fish bladders

**Gelatin**



derived from collagen of an animal. i.e. skin, boiled crushed horns and hoofs, connective tissues, organs, and some intestines. Generally from cattle, chicken, pigs, and horses

**Casein**



a protein found in cows milk

himmeeand123.com









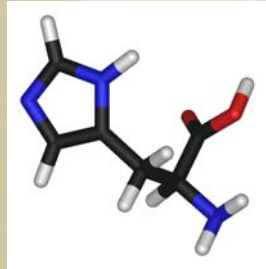
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<b>Problem</b>	<b>Risk group</b>	<b>Solution (HACCP)</b>
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Biogenic Amines	allergic people (Ex: Histamine)	

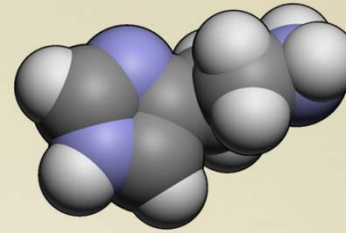
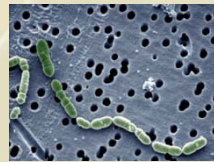
# Biogenic Amines Problem

## Origin



Histidine

Decarboxylase  
enzymes



Histamine

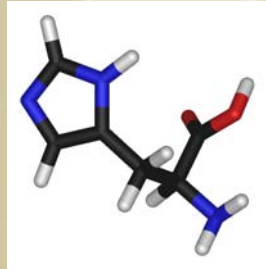
Others:

- Tyramine
- Putrescine
- Cadaverine
- Phenylethylamine

**Lactic Bacteria** (*O. Oeni*) metabolism => **Biogenic Amines** ↑

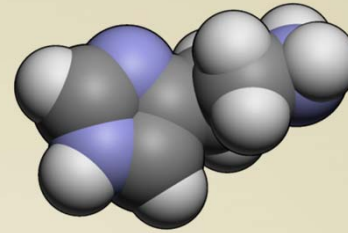
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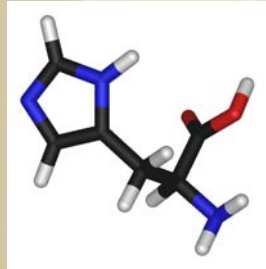
**Lactic Bacteria (*O. Oeni*) metabolism => Biogenic Amines ↑**

## Real Group of Risk

**Allergic People => Biogenic Amines + Alcohol**

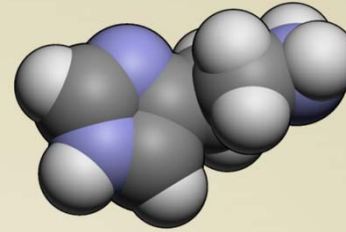
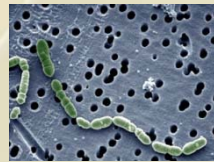
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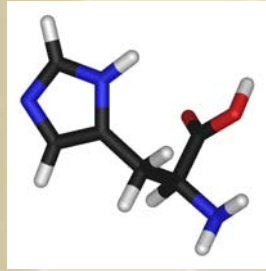
**Alergic People => Biogenic Amines + Alcohol**

## Legal Limits

**Germany, Switzerland, Belgium, Austria, Holland, France =>  
2-10 mg/L (Histamine)**

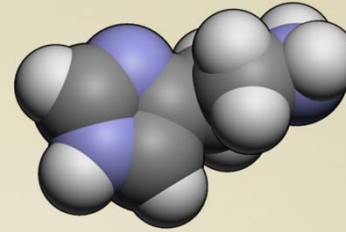
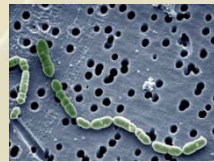
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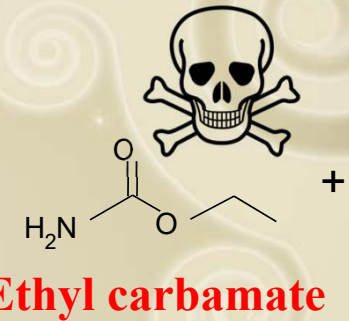
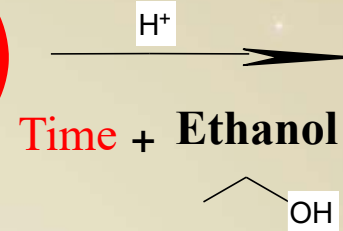
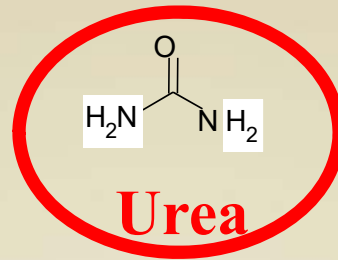
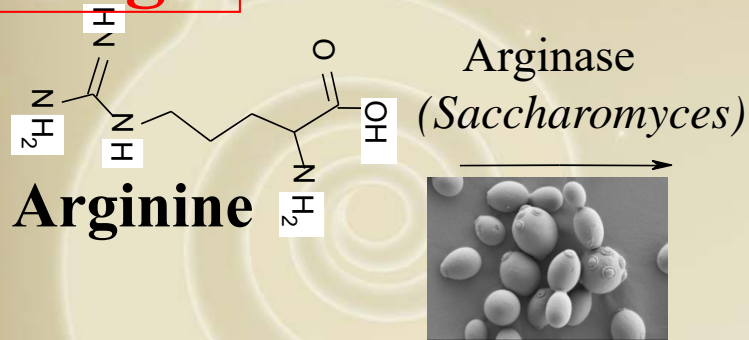
**Germany, Switzerland, Belgium, Austria, Holland, France =>  
2-10 mg/L (Histamine)**

**How should we control it ?**

<b>Problem</b>	<b>Risk group</b>	<b>Solution (HACCP)</b>
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
SO <sub>2</sub>	Asthmatics	Labeled: It contains sulfites. Other preservatives (sulfites free) Legal limits
Allergenic additives	allergic people (Ex: albumin)	Labeled. Or non allergenic additives
Biogenic Amines	allergic people (Ex: Histamine)	<b>legal limits =&gt;</b> <b>but no viable industrial solution ?</b>
Ethyl Carbamate	Anyone (carcinogenic)	

# Ethyl Carbamate Problem

## Origin

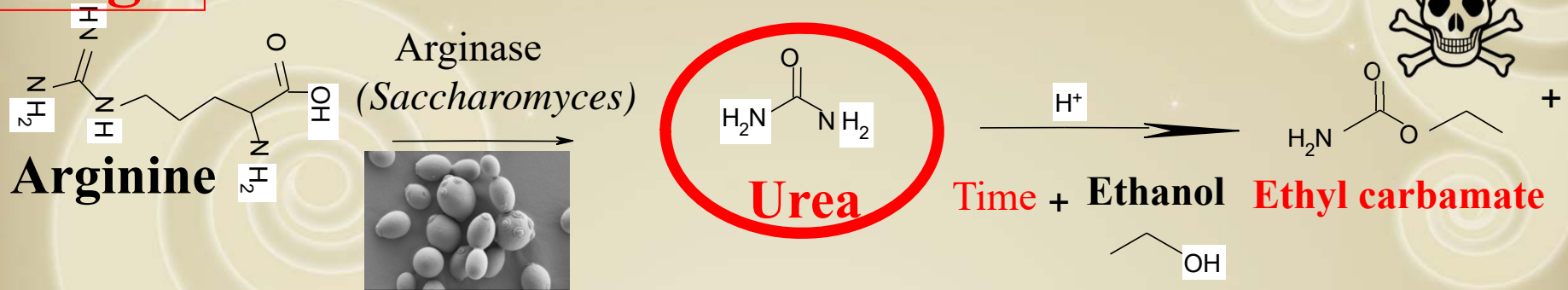


**Yeast metabolism => Ethyl Carbamate Precursor ↑ (UREA)**

Uthurry et al.2004; Bertrand 1993

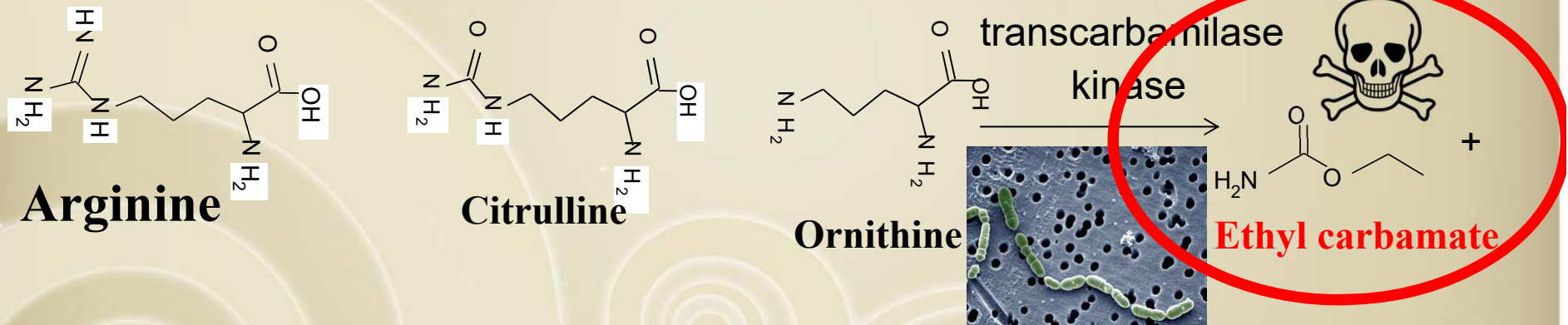
# Ethyl Carbamate Problem

## Origin



**Yeast** metabolism  $\Rightarrow$  Ethyl Carbamate Precursor  $\uparrow$  (UREA)

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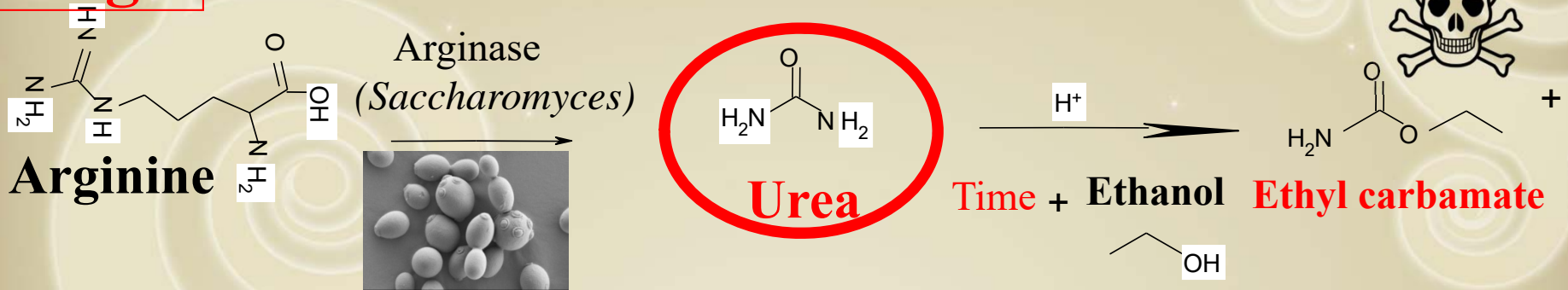
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Tegmo-Larsson et al.1989



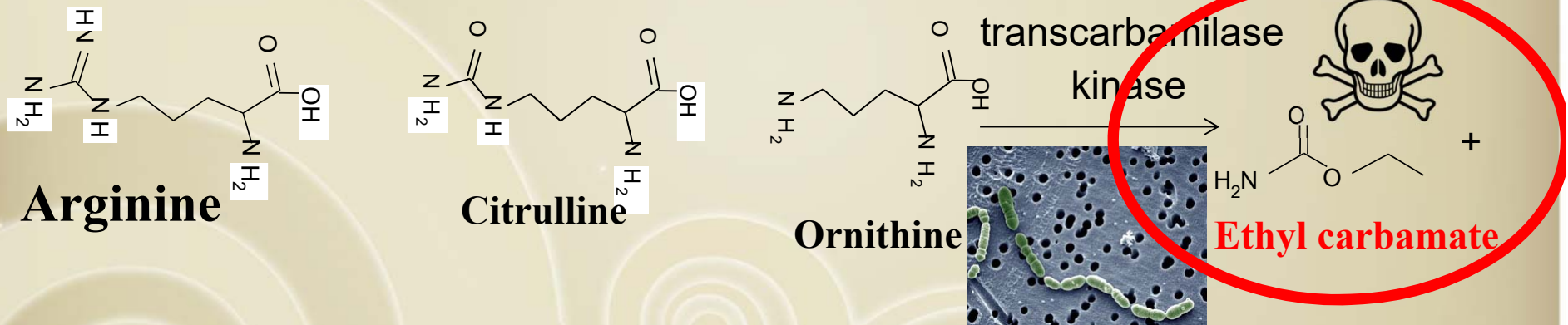
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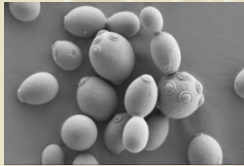
## Legal Limits

Canada, USA (recomendation) and Japan  $\Rightarrow$  **15-30  $\mu\text{g/L}$**

# Ethyl Carbamate Industrial Situation

## Origin

**Yeast** metabolism => Ethyl Carbamate **Precursor** ↑ (UREA)



Uthurry et al.2004; Bertrand 1993

**Lactic Bacteria** (*O. Oeni*) metabolism => Ethyl Carbamate ↑



Tegmo-Larsson et al.1989

## Legal Limits

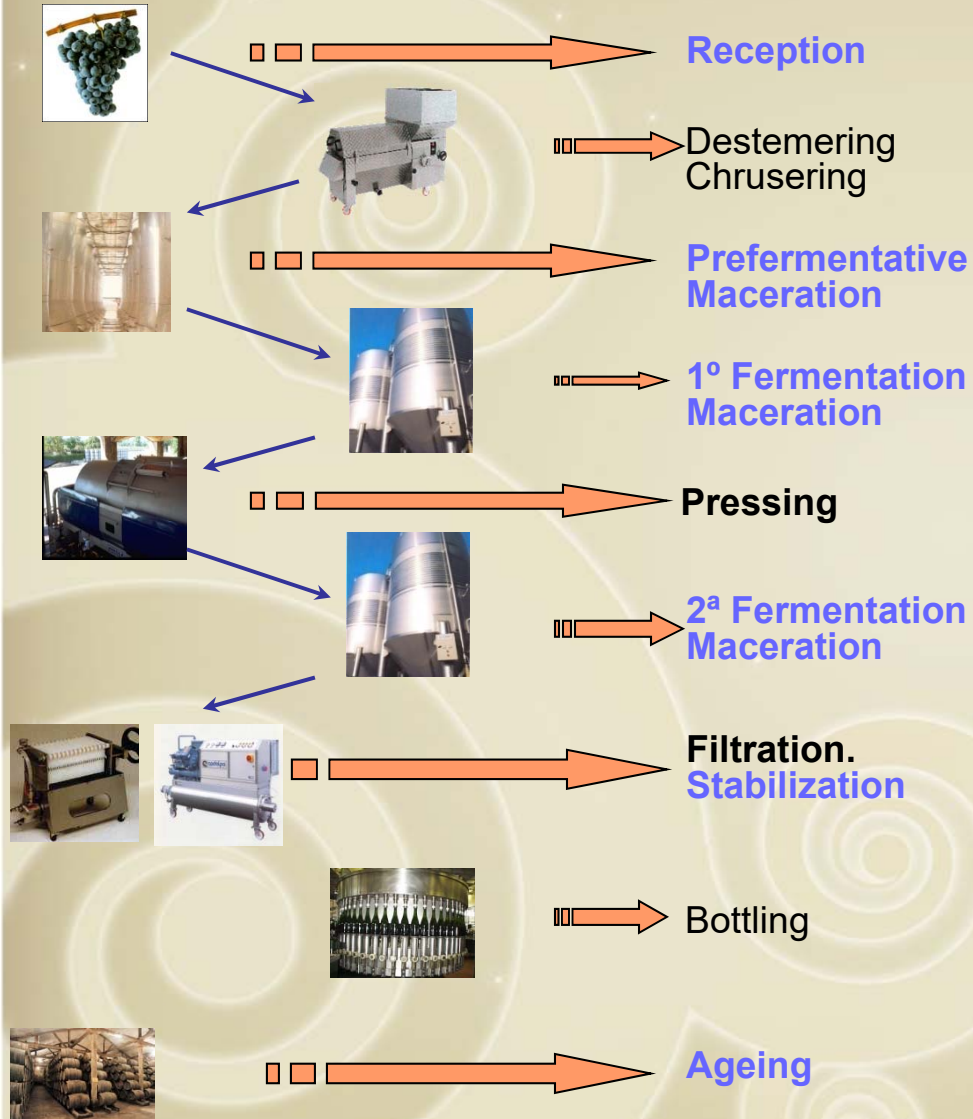
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**How should we control it ?**

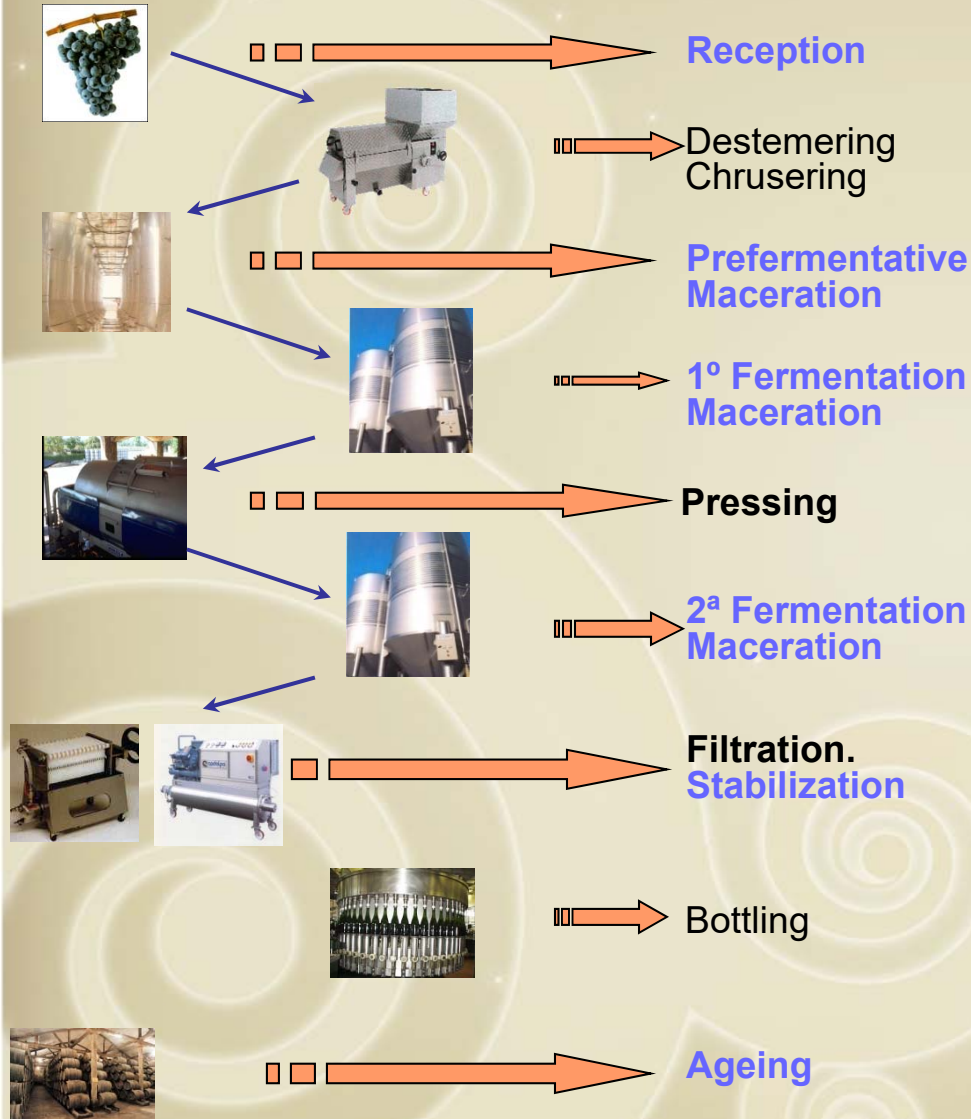
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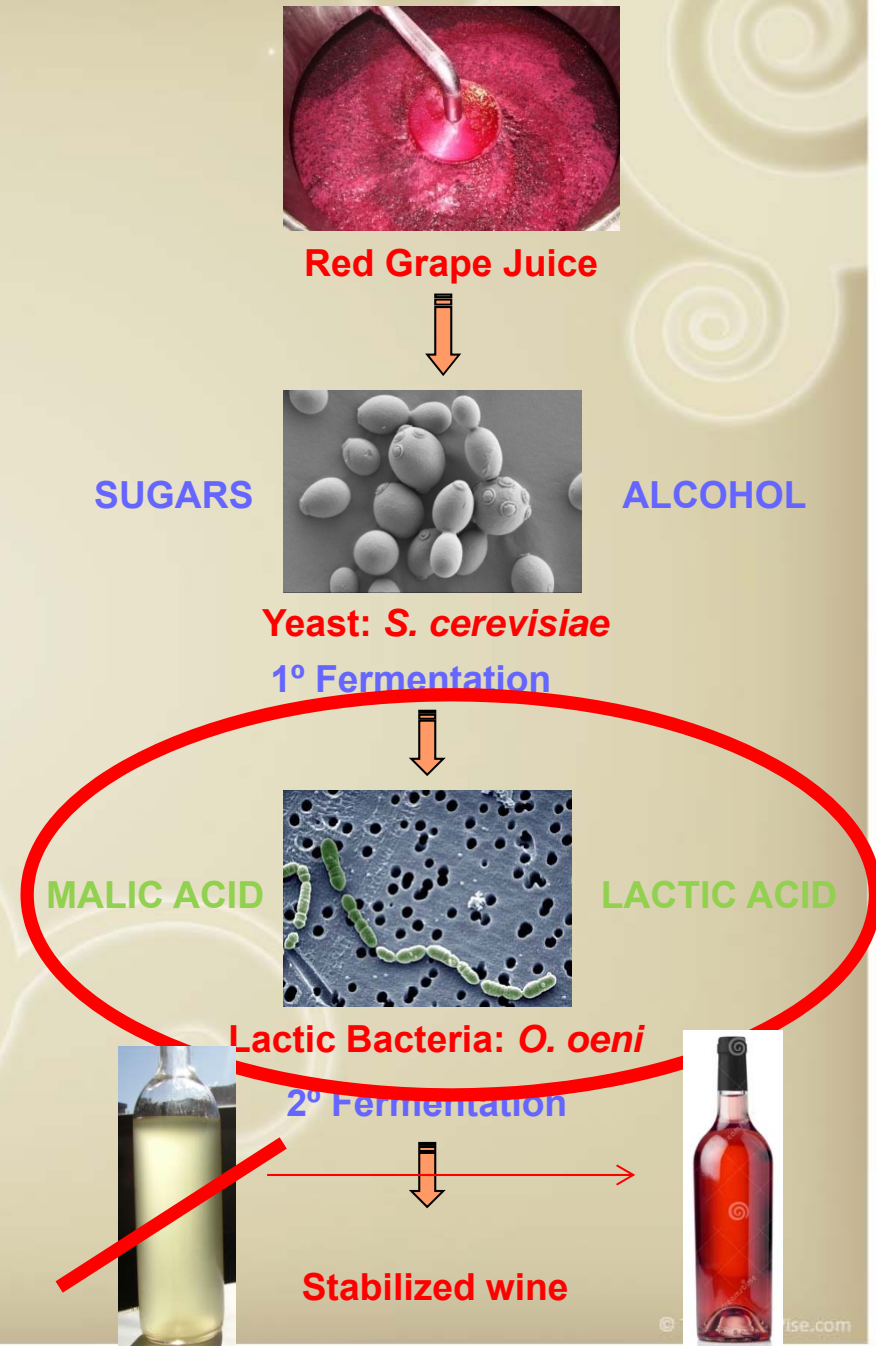
# PRODUCTION OF RED WINE



# PRODUCTION OF RED WINE



# MICROBIOLOGICAL SIMPLIFICATION



# INDUSTRIAL ALTERNATIVE PROPOSAL



Red Grape Juice



SUGARS

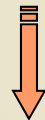
MALIC ACID



ALCOHOL

ETHANOL

Yeast: *Schizo. pombe*  
Only 1° Fermentation



Stabilized wine



We avoid possible collateral effects related to 2° Fermentation by Lactic Bacteria:

- Biogenic amines ↓
- Ethyl carbamate ↓

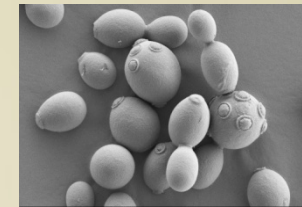
# MICROBIOLOGICAL SIMPLIFICATION



Red Grape Juice



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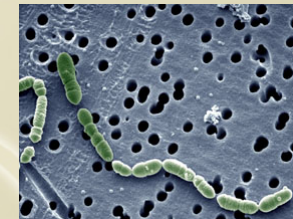


ALCOHOL

Yeast: *S. cerevisiae*  
1° Fermentation



MALIC ACID



LACTIC ACID

Lactic Bacteria: *O. oeni*  
2° Fermentation

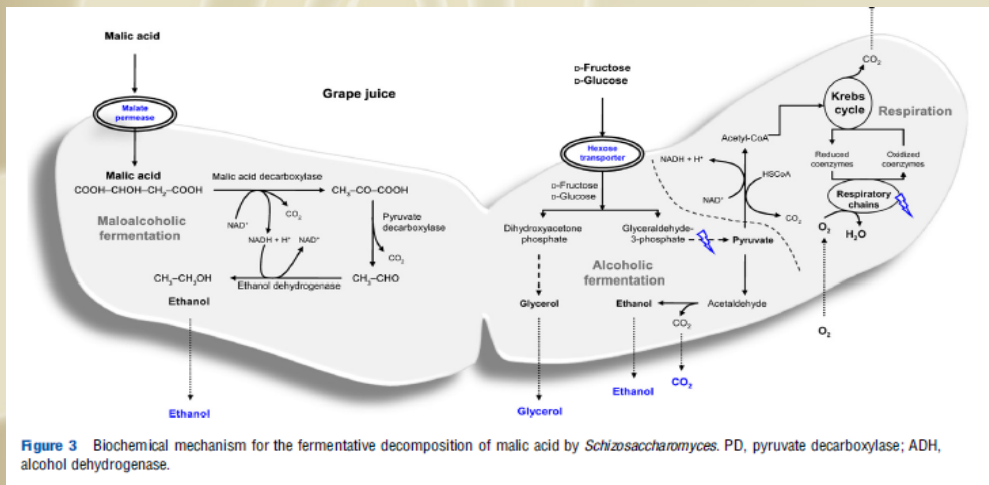


Stabilized wine



# Why to use *Schizosaccharomyces* selected strains?

- Classic use => deacidification (Malic Acid ↓)
  - Recommended Practice by International Organization of Vine and Wine.
  - **Alternative to MaloLactic fermentation** by Bacteria (**Collateral effects ↓**).



Benito et al 2012; Benito et al 2014;  
Benito et al 2015

- **Urease Activity** => **Urea ↓** (Main Ethyl Carbamate precursor ↓)

*Urease-positive* species of yeast. Deák 2008.

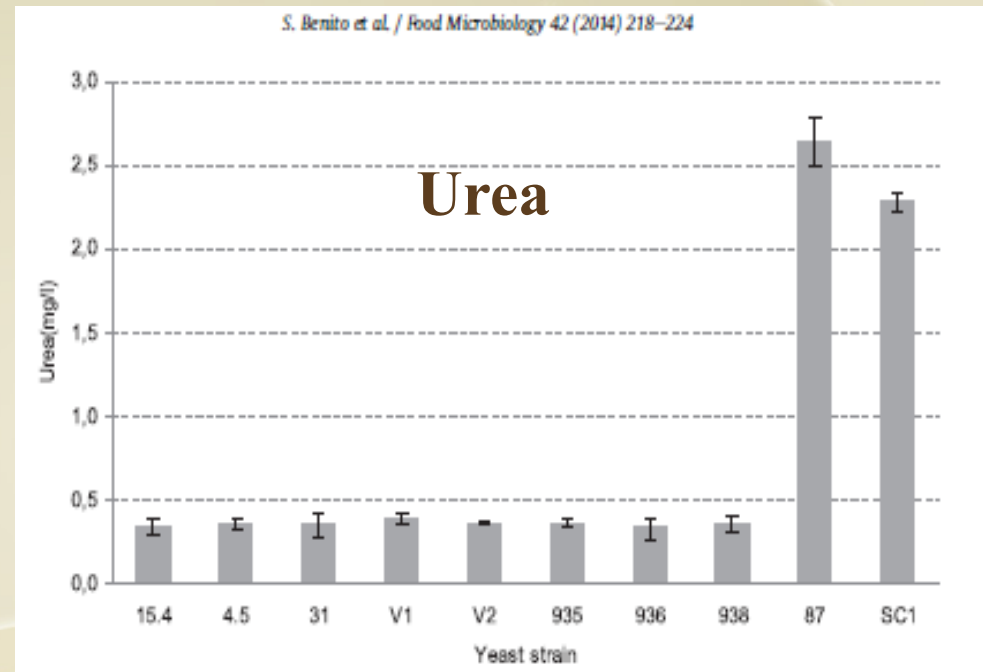
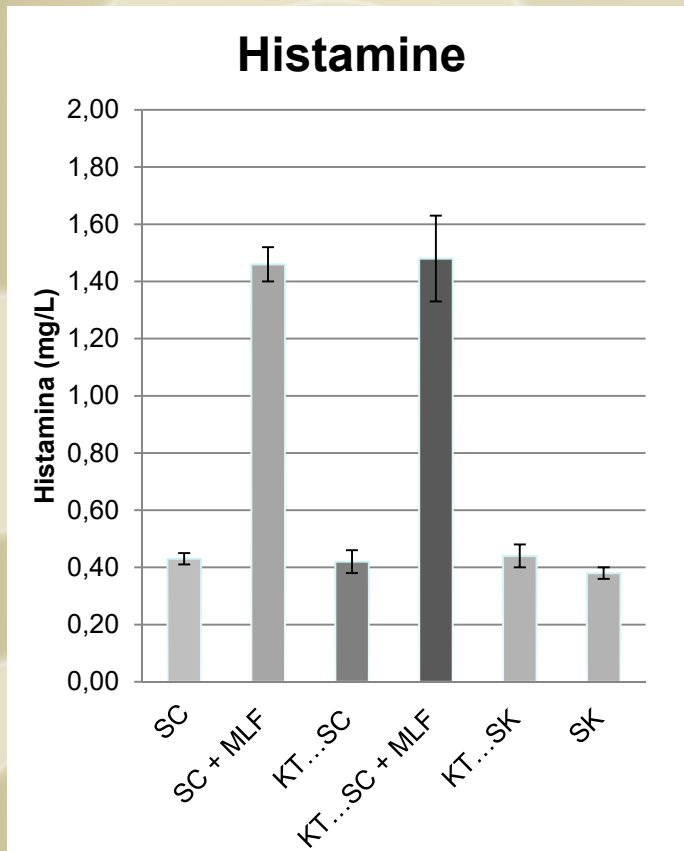
<i>Bulleromyces albus</i>	<i>Filob capsuligenum</i>	<i>Schizo. japonicus</i>
<i>Cry.albidus</i>	<i>F'ella neoformans</i>	<i>Schizo. octosporus</i>
<i>Cry. curvatus</i>	<i>Leucosp scotti</i>	<i>Schizo. pombe</i>
<i>Cry. diffyuens</i>	<i>Moniliella suaveolens</i>	<i>Spori. pararoseus</i>
<i>Cry. humicolus</i>	<i>Rho glutinis</i>	<i>Trisp. moniliforme</i>
<i>Cry.laurentii</i>	<i>Rho minuta</i>	<i>Guehom.pullulans</i>
<i>Cystofilob</i>	<i>Rho mucilaginoso</i>	

Lubbers et al. 1996; Déak.2008; Benito et al 2014

**To avoid Biogenic Amines and E. Carbamate ?**

# Classical Fermentations $\Leftrightarrow$ Schizo Fermentations

*S. cerevisiae* + *O. oeni*                      Selected *S.pombe*



[Benito et al. 2014. Food Microbiology. 42: 218-224](#)

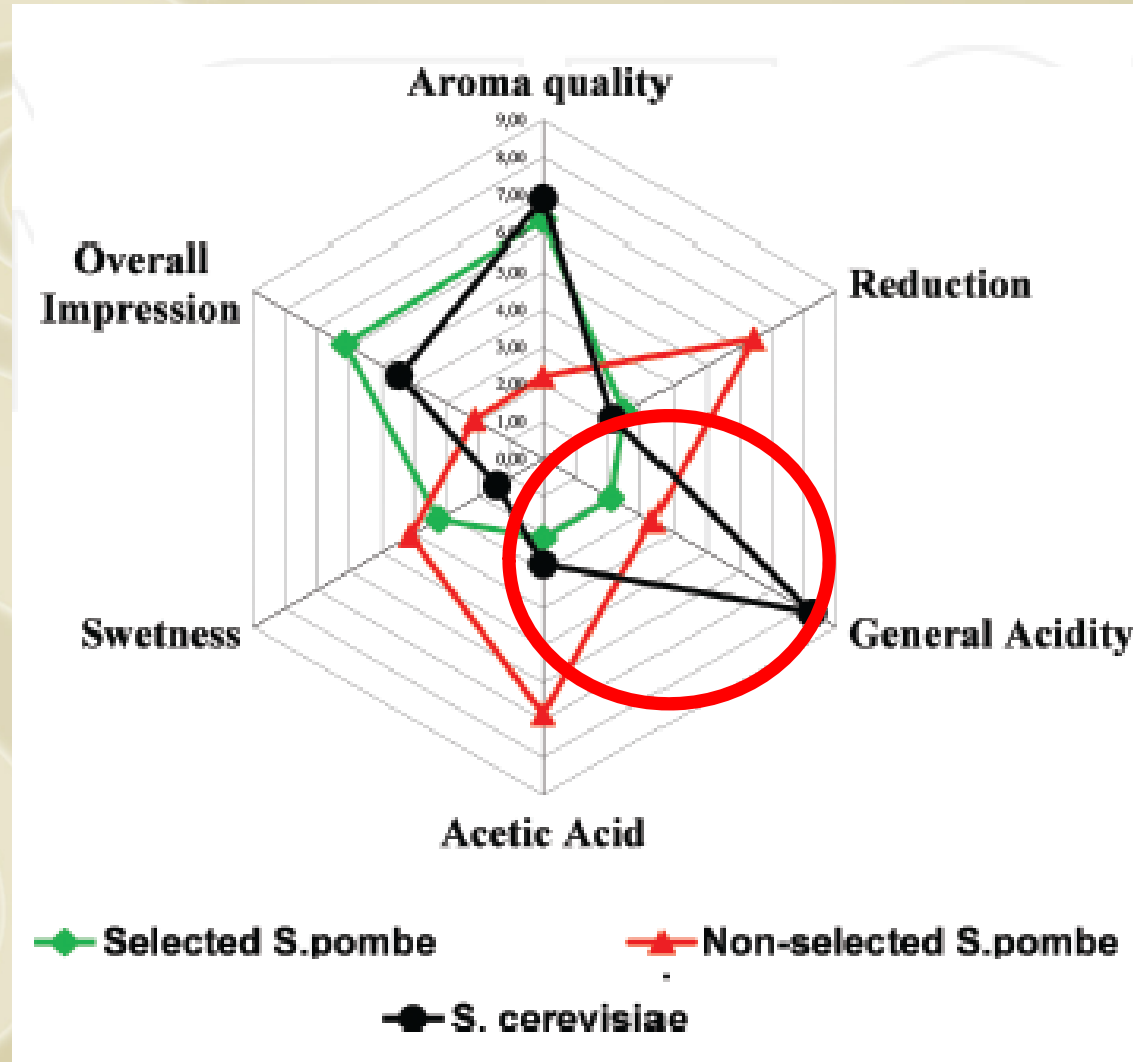
[Benito et al. 2015. Molecules. 20: 9510-9523](#)

**Lower levels of Biogenic Amines and Urea in fermentations involving selected strains of *S.pombe*.**





Main sensory problem => Lack of acidity => Malic acid ↓



# South Spanish vineyard problems

# South spanish vineyard problems

Warm Viticulture area (Global climate change)



# South spanish vineyard problems

Warm Viticulture area (Global climate change)

Grape composition:

- Sugar content ↑
- Acidity ↓



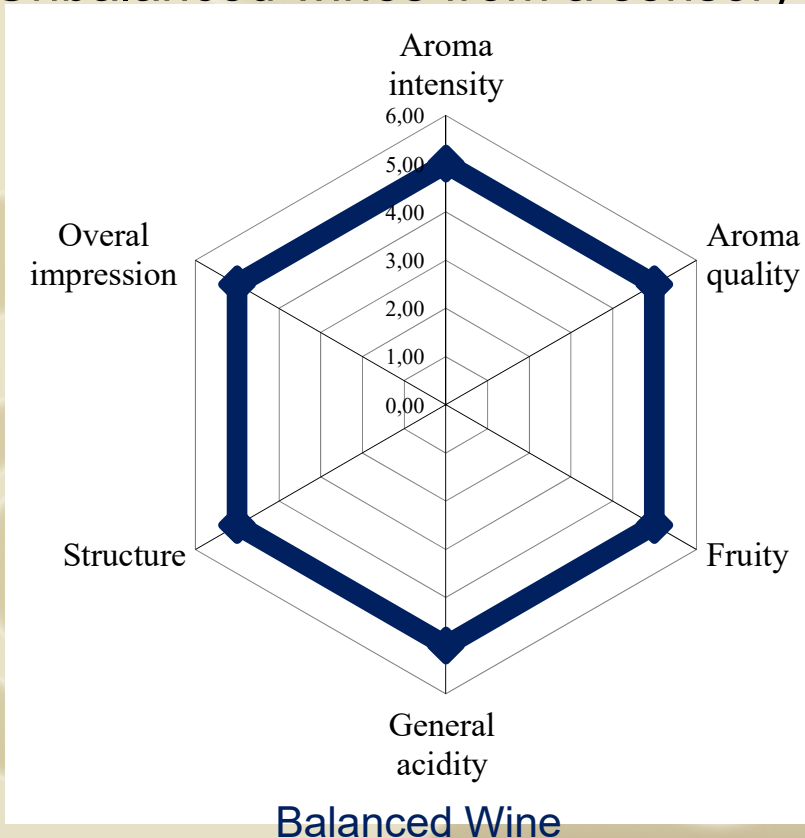
Unbalanced wines from a sensory point of view.

# South spanish vineyard problems

Grape composition:

- Sugar content ↓
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Unbalanced wines from a sensory point of view.



Unalanced Wine

# South spanish vineyard problems

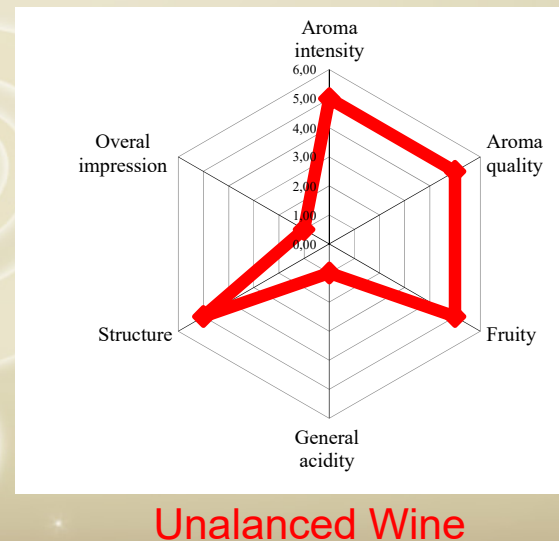
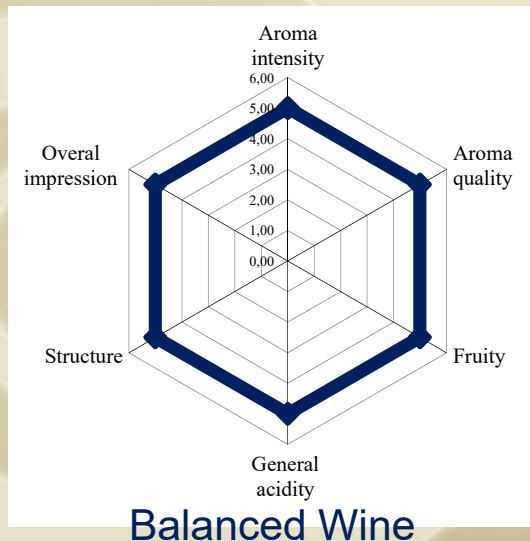
Warm Viticulture area (Global climate change)

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Unbalanced wines from a sensory point of view.



# South spanish vineyard problems

## Possible Solutions to avoid lack of acidity

Acidification with legal industrial acids (food grade).

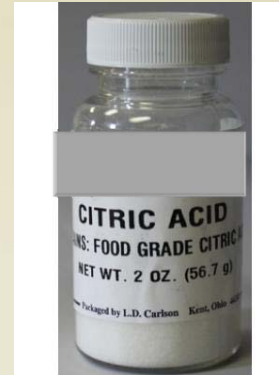
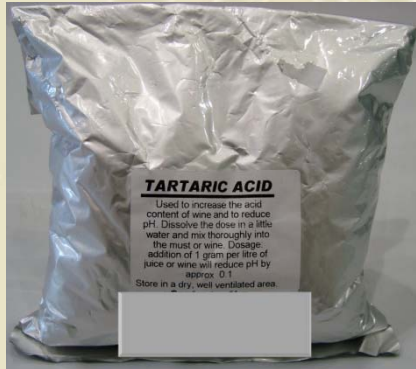




# South spanish vineyard problems

## Possible Solutions to avoid lack of acidity

Acidification with legal industrial acids (food grade).



Disadvantages:

Acid stability

Legal limits 1-2 g/l depending on the used acid.

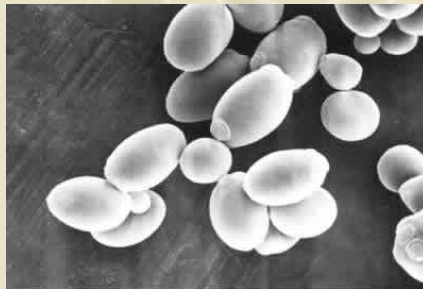
Cost of food grade acids

# South spanish vineyard problems

## Possible Solutions to avoid lack of acidity

Microbiological solution => Use of *Lachancea thermotolerans*

[www.diark.org](http://www.diark.org)



Non - <i>Saccharomyces</i> Yeast specie	Main Industrial application
<i>Torulaspora delbrueckii</i>	Body ↑ softness ↑ roundness ↑ Aroma complexity ↑ volatile acidity ↓
<i>Pichia kluyveri</i>	Aroma complexity ↑ (fruit character). Precursors liberation => grape variety character ↑
<i>Metschnikowia pulcherrima</i>	Aroma complexity ↑ (fruit character). Precursors liberation => grape variety character ↑
<i>Hansenula anomala</i>	High stable color compounds ↑ (red wine)
<i>Schizosaccharomyces pombe</i>	Acidity ↓ Food Safety ↑
<i>Lachancea thermotolerans</i>	Acidity ↑ volatile acidity ↓

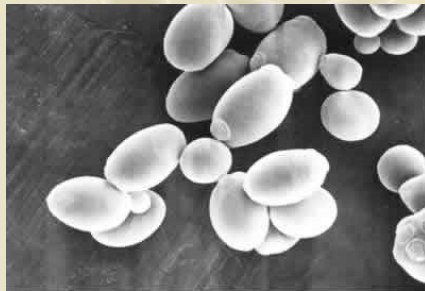
Benito, A., Calderón, F., Palomero, F., & Benito, S. (2016). Quality and composition of Airen wines fermented by sequential inoculation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae*. Food Technol Biotechnol, 54.

# South spanish vineyard problems

## Possible Solutions to avoid lack of acidity

Microbiological solution => Use of *Lachancea thermotolerans*

[www.diark.org](http://www.diark.org)



Advantages:

Increases of Lactic acid about 1-3 g/L for regular inoculations.

Disadvantages:

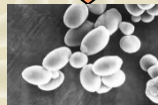
*L. Thermotolerans* is not able to complete a regular fermentation process (So. a *S. cerevisiae*/*S. pombe* partner is needed to properly finish).

Not very resistant to SO<sub>2</sub>.

## NEW ALTERNATIVE PROPOSAL



Red Grape Juice



LACTIC ACID

Yeast: *Lach. thermotolerans*

SUGARS

MALIC ACID

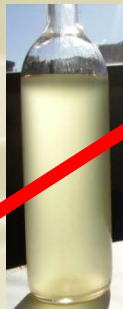


ALCOHOL

ETHANOL

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Only 1° Fermentation



Stabilized wine



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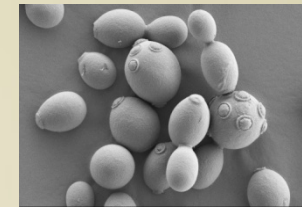
- Biogenic amines ↓
- Ethyl carbamate ↓

Acidity ↑

## CLASSIC WINEMAKING



Red Grape Juice

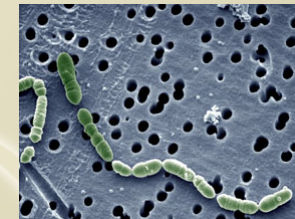


SUGARS

ALCOHOL

Yeast: *S. cerevisiae*

1° Fermentation

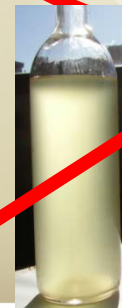


MALIC ACID

LACTIC ACID

Lactic Bacteria: *O. oeni*

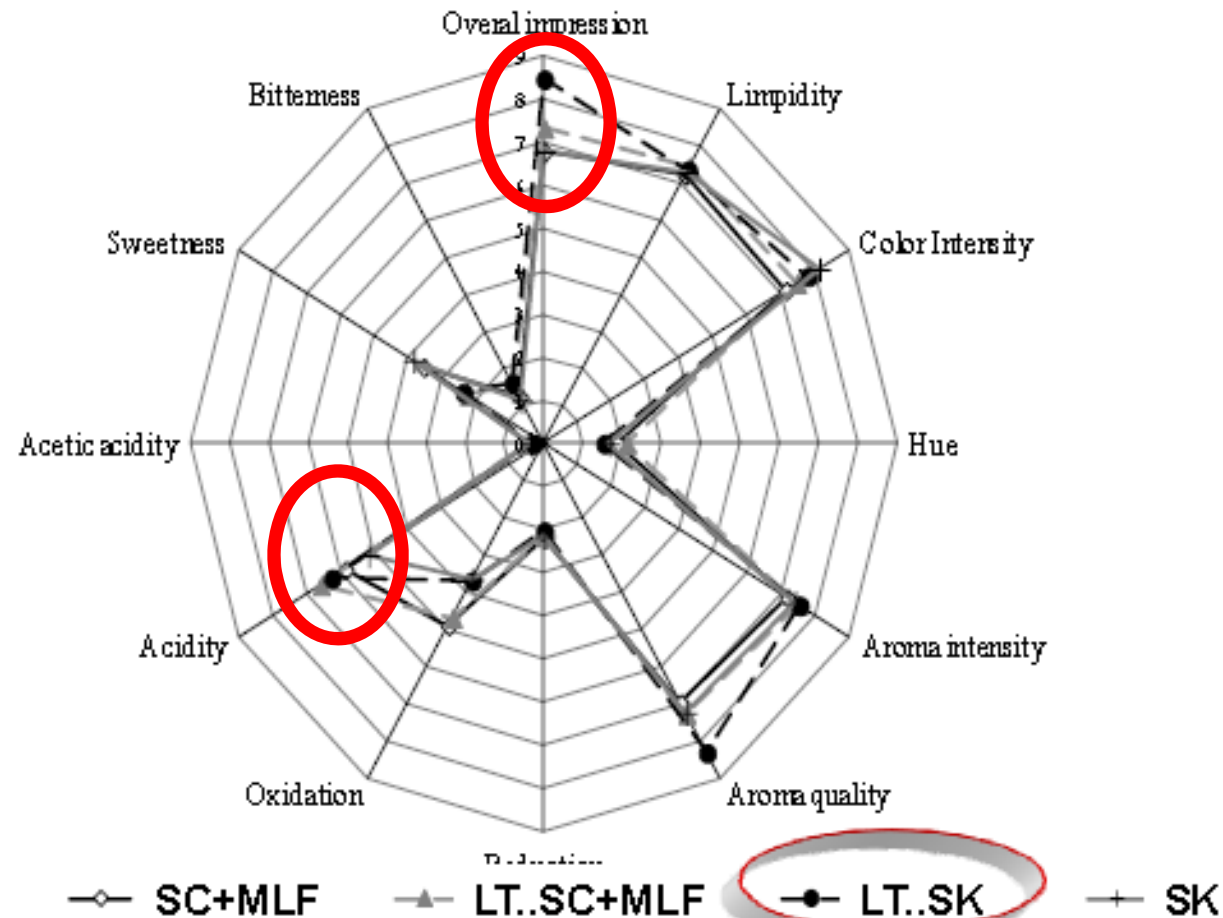
2° Fermentation



Stabilized wine



# Sensory Analysis



Benito A, Palomero F, Calderón F, Benito S (2015) Combine Use of Selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* Yeast Strains as an Alternative to the Traditional Malolactic Fermentation in Red Wine Production. *Molecules* 20:9510–9523.

# Conclusions

- Most wine/Food Safety problems have a relatively easy solution.
- Using *Schizosaccharomyces pombe* fermentation technology is possible to control two specific wine/Food Safety problems that are more complex:
  - Biogenic amines. => Specific consumers
  - Ethyl Carbamate. => Specific markets
- Using combined *L. thermotolerans* and *S. pombe* fermentations technology is possible to increase wine quality increasing the wine acidity (main collateral effect about using *S.pombe* in low acidic grape juices).

# Bibliography

Food Microbiology 42 (2014) 218–224



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## Selection of appropriate *Schizosaccharomyces* strains for winemaking

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## Use of *Schizosaccharomyces* strains for wine fermentation—Effect on the wine composition and food safety

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Article

## Combine Use of Selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* Yeast Strains as an Alternative to the Traditional Malolactic Fermentation in Red Wine Production

Ángel Benito, Fernando Calderón, Felipe Palomero and Santiago Benito \*

PLOS ONE

RESEARCH ARTICLE

## Selected *Schizosaccharomyces pombe* Strains Have Characteristics That Are Beneficial for Winemaking

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Recommended Practice by International Organization of Vine and Wine.

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# Bibliography

- Benito S, Palomero P, Calderón F, Palmero D, Suárez-Lépe JA (2014) Selection of Appropriate *Schizosaccharomyces* strains for winemaking. *Food Microbiology* 42:218-224
- Benito A, Palomero F, Calderón F, Benito S (2015) Combine Use of Selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* Yeast Strains as an Alternative to the Traditional Malolactic Fermentation in Red Wine Production. *Molecules* 20:9510–9523
- Benito A, Jeffares D, Palomero F, Calderón F, Bai FY, Bähler J, Benito S (2016) Selected *Schizosaccharomyces pombe* Strains Have Characteristics That Are Beneficial for Winemaking. *PLoS one*, 11(3), e0151102.
- Mylona AE, Del Fresno JM, Palomero F, Loira I, Bañuelos MA, Morata A, Calderón F, Benito S, Suárez-Lepe JA (2016) *International Journal of Food Microbiology*. 232: 63-72.
- Benito, S., Hofmann, T., Laier, M., Lochbühler, B., Schüttler, A., Ebert, K., ... & Rauhut, D. (2015). Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-*Saccharomyces* and *Saccharomyces cerevisiae*. *European Food Research and Technology*, 241(5), 707-717.
- Benito, A., Calderón, F., Palomero, F., & Benito, S. (2016). Quality and composition of Airen wines fermented by sequential inoculation of *Lachancea thermotolerans* and *Saccharomyces cerevisiae*. *Food Technol Biotechnol*, 54.