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?
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
- SO2 or other preservatives
- Additives (Clarification)
- Biogenic Amines
- Ethyl Carbamate
## Summary
### Main Wine Food Safety problems <=> Solutions

<table>
<thead>
<tr>
<th>Problem</th>
<th>Risk group</th>
<th>Solution (HACCP)</th>
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<tbody>
<tr>
<td>Ethanol</td>
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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)
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![Image of labeled alcohol wine]

Responsible Consumers
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![Diagram of filtration process](image-url)
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**IS YOUR WINE VEGAN?**

- **albumin**
- **isinglass**
- **Casein**
- **Gelatin**

Use of new additives or technologies
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Biogenic Amines Problem

**Origin**

- Histidine → Histamine

 shred Decarboxylase enzymes

Other biogenic amines:
- Tyramine
- Putrescine
- Cadaverine
- Phenylethylamine

Lactic Bacteria (*O. Oeni*) metabolism $\Rightarrow$ Biogenic Amines
Biogenic Amines Problem

Origin

Histidine → Decarboxylase enzymes → Histamine

Others: -Tyramine -Putrescine -Cadaverine -Phenylethylamine

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

Real Group of Risk

Alergic People → Biogenic Amines + Alcohol
Biogenic Amines Problem

Origin

Histidine \rightarrow \text{Decarboxylase enzymes} \rightarrow \text{Histamine}

Others:
- Tyramine
- Putrescine
- Cadaverine
- Phenylethylamine

Lactic Bacteria (\textit{O. Oeni}) metabolism \rightarrow \text{Biogenic Amines} ↑

Real Group of Risk

Alergic People \rightarrow \text{Biogenic Amines + Alcohol}

Legal Limits

Germany, Switzerland, Belgium, Austria, Holland, France \rightarrow
2-10 mg/L (Histamine)
Biogenic Amines Problem

Origin

Histidine \xrightarrow{Decarboxylase enzymes} \text{Histamine}

Others:
- Tyramine
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Lactic Bacteria (O. Oeni) metabolism $\Rightarrow$ Biogenic Amines $\uparrow$

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How should we control it?
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Ethyl Carbamate Problem

Origin

Arginine (Saccharomyces)

Arginase

Urea

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

Uthurray et al. 2004; Bertrand 1993
Ethyl Carbamate Problem

**Origin**

Arginine

\[
\text{Arginase (Saccharomyces)} \quad \text{Arginine} \quad \text{Urea} \quad \text{Ethanol} \quad \text{Ethyl carbamate}
\]

\[\text{Time} + \text{Ethanol} \quad \text{Ethyl carbamate}\]

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

Uthurry et al.2004; Bertrand 1993

Arginine

Citrulline

Ornithine

Lactic Bacteria (O. Oeni) metabolism $\Rightarrow$ Ethyl Carbamate $\uparrow$

Tegmo-Larsson et al.1989
**Ethyl Carbamate Problem**

**Origin**

Arginine → Arginase (Saccharomyces) → Urea → Time + Ethanol → Ethyl carbamate

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

Uthurry et al. 2004; Bertrand 1993

Arginine → Citrulline → Ornithine → deiminase → transcarbamylase → kinase → Ethyl carbamate

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

Tegmo-Larsson et al. 1989

**Legal Limits**

Canada, USA (recommendation) and Japan => 15-30 µg/L
Ethyl Carbamate Industrial Situation

**Origin**

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

Uthurry et al. 2004; Bertrand 1993

*Lactic Bacteria* (*O. Oeni*) metabolism $\Rightarrow$ Ethyl Carbamate $\uparrow$

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PRODUCTION OF RED WINE

1º Fermentation
Maceration
Filtration.
Stabilization
Ageing
Pressing
Bottling
Destemering
Chrusering

Fermentation
Maceration

Prefermentative Maceration
1º Fermentation Maceration
Pressing
2º Fermentation Maceration
Filtration.
Stabilization
Bottling
Ageing
**PRODUCTION OF RED WINE**

- Reception
- Destemering Chrusering
- Prefermentative Maceration
- Pressing
- 1º Fermentation Maceration
- 2º Fermentation Maceration
- Filtration. Stabilization
- Bottling
- Ageing

**MICROBIOLOGICAL SIMPLIFICATION**

- Red Grape Juice
- SUGARS
- Yeast: *S. cerevisiae*
- ALCOHOL
- 1º Fermentation
- MALIC ACID
- LACTIC ACID
- Lactic Bacteria: *O. oeni*
- 2º Fermentation
- Stabilized wine

Yeast:

- *S. cerevisiae*
- *O. oeni*
**INDUSTRIAL ALTERNATIVE PROPOSAL**

Red Grape Juice

SUGARS

MALIC ACID

Yeast: *Schizo. pombe*

Only 1º Fermentation

ALCOHOL

ETHANOL

Stabilized wine

**MICROBIOLOGICAL SIMPLIFICATION**

Red Grape Juice

SUGARS

MALIC ACID

Yeast: *S. cerevisiae*

1º Fermentation

ALCOHOL

LACTIC ACID

Lactic Bacteria: *O. oeni*

2º Fermentation

Stabilized wine

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

- Biogenic amines ↓
- Ethyl carbamate ↓
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 ↓).

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

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<td>Bulleromyces aibus</td>
<td>Filob capsuligenum</td>
<td>Schiz. japonicus</td>
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<tr>
<td>Cry. albicus</td>
<td>F. olla neoformans</td>
<td>Schiz. octosporus</td>
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<tr>
<td>Cry. curvatus</td>
<td>Leucos. scottii</td>
<td>Schiz. pombe</td>
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<tr>
<td>Cry. diffluens</td>
<td>Monil. suaveolens</td>
<td>Spori. pararosaeus</td>
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<td>Cry. humicolus</td>
<td>Rho glutinis</td>
<td>Trisp. moniliforme</td>
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<tr>
<td>Cry. laurientii</td>
<td>Rho minuta</td>
<td>Gueh. pullulans</td>
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<tr>
<td>Cystofilob</td>
<td>Rho mucilaginosa</td>
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To avoid Biogenic Amines and E. Carbamate?
Lower levels of Biogenic Amines and Urea in fermentations involving selected strains of *S. pombe*.

Benito et al. 2014. *Food Microbiology*. 42: 218-224

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 ↓
- Acidity ↓

Unbalanced wines from a sensory point of view.
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Warm Viticulture area (Global climate change)

Grape composition:
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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
Microbiological solution => Use of *Lachancea thermotolerans*

www.diark.org

<table>
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<tr>
<th>Non-Saccharomyces Yeast specie</th>
<th>Main Industrial application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torulaspora delbrueckii</td>
<td>Body ↑, softness ↑, roundness ↑, Aroma complexity ↑, volatile acidity ↓</td>
</tr>
<tr>
<td>Pichia kluvyeri</td>
<td>Aroma complexity ↑ (fruit character), Precursors liberation ⇒ grape variety character ↑</td>
</tr>
<tr>
<td>Metschnikowia pulcherrima</td>
<td>Aroma complexity ↑ (fruit character), Precursors liberation ⇒ grape variety character ↑</td>
</tr>
<tr>
<td>Hansenula anomala</td>
<td>High stable color compounds ↑ (red wine)</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>Acidity ↓, Food safety ↑</td>
</tr>
<tr>
<td><em>Lachancea thermotolerans</em></td>
<td>Acidity ↑, volatile acidity ↓</td>
</tr>
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South spanish vineyard problems
Possible Solutions to avoid lack of acidity

Microbiological solution => Use of *Lachancea thermotolerans*

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* partener is needed to properly finish).

Not very resistant to SO₂.
NEW ALTERNATIVE PROPOSAL

Red Grape Juice

Yeast: *Lach. thermotolerans*

SUGARS → LACTIC ACID

Only 1º Fermentation

Stabilized wine

We avoid possible collateral effects related to 2º Fermentation by Lactic Bacteria:
- Biogenic amines ↓
- Acidity ↑
- Ethyl carbamate ↓

CLASSIC WINEMAKING

Red Grape Juice

Yeast: *S. cerevisiae*

1º Fermentation

SUGARS → ALCOHOL

MALIC ACID → ETHANOL

Lactic Bacteria: *O. oeni*

2º Fermentation

MALIC ACID → LACTIC ACID

Stabilized wine
Sensory Analysis

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).
Selection of appropriate *Schizosaccharomyces* strains for winemaking

S. Benito*, F. Palomero, F. Calderón, D. Palmero, J.A. Suárez-Lepe

Dept. Tecnología de Alimentos, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain

*Molecules* 2015, 20, 9510-9523; doi:10.3390/molecules20069510

**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

**Recommended Practice by International Organization of Vine and Wine.**
Bibliography


