

August 18, 2015

The use of *Schizosaccharomyces* yeast in order to reduce the content of Biogenic Amines and Ethyl Carbamate in wines



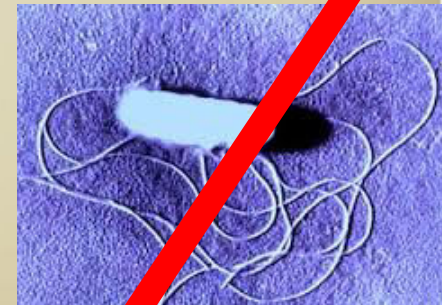
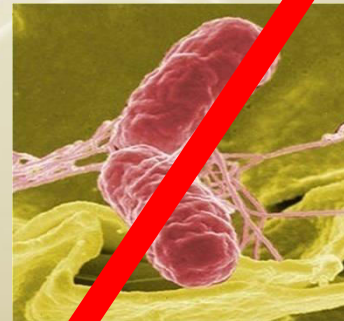
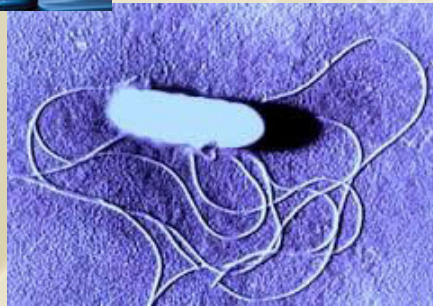
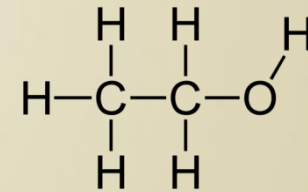
Dept. Chemistry and Food Technology
Prof. Santiago Benito Sáez.

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.
- .

Introduction

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



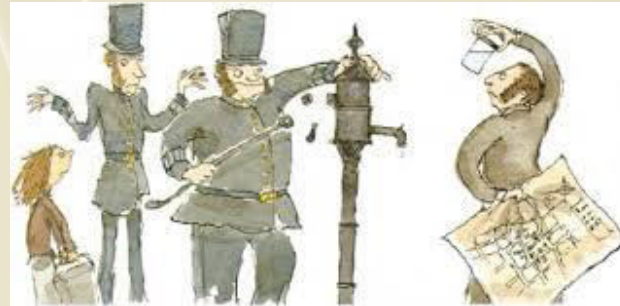
Introduction

London 1854 Broad Street (Cholera outbreak)

What would you prefer to drink ?

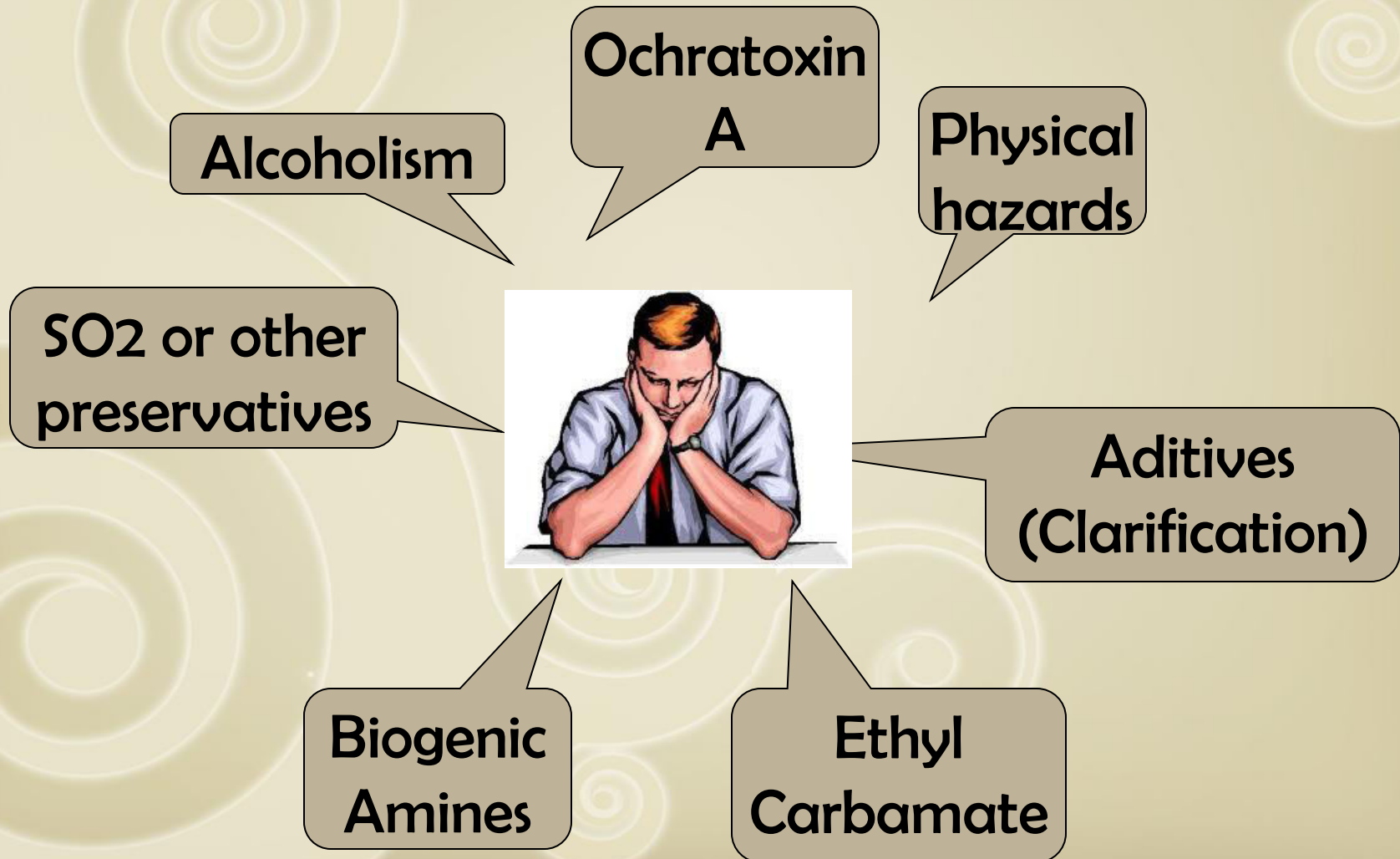


NOTICE.
PREVENTIVES OF
CHOLERA!
Published by order of the Sanatory Committee, under the sanction of the Medical Council.
BE TEMPERATE IN EATING & DRINKING!
Avoid Raw Vegetables and Unripe Fruit !.
Abstain from COLD WATER, when heated, and above all from *Ardent Spirits,* and if habit have rendered them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



Summary

Main Wine Food Safety problems <=> Solutions

Problem	Risk group	Solution (HACCP)
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

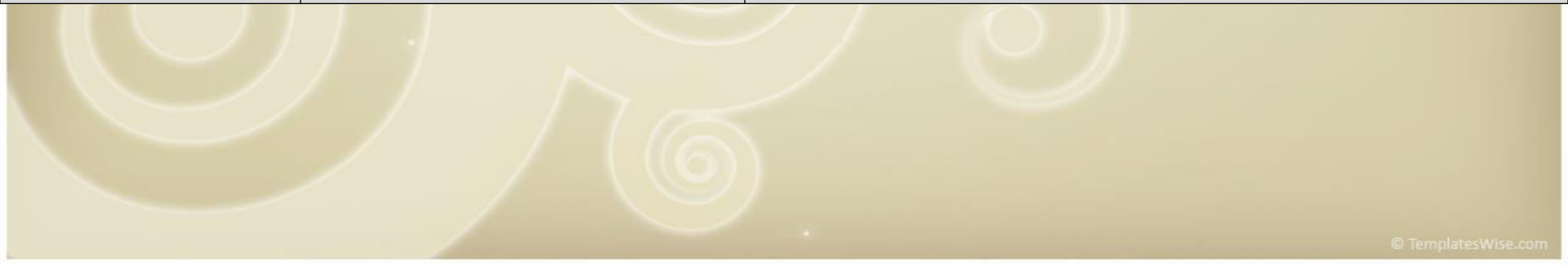
Alcoholics,
diabetics.....

Labeled
Free alcohol wine



Responsible Consumers

Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	



Problem

Risk group

Solution (HACCP)

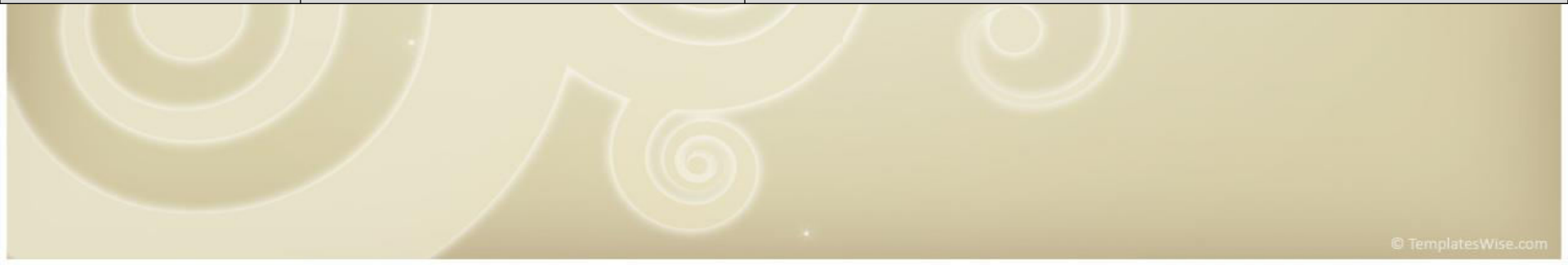
Physical hazards

Anyone

Filtration



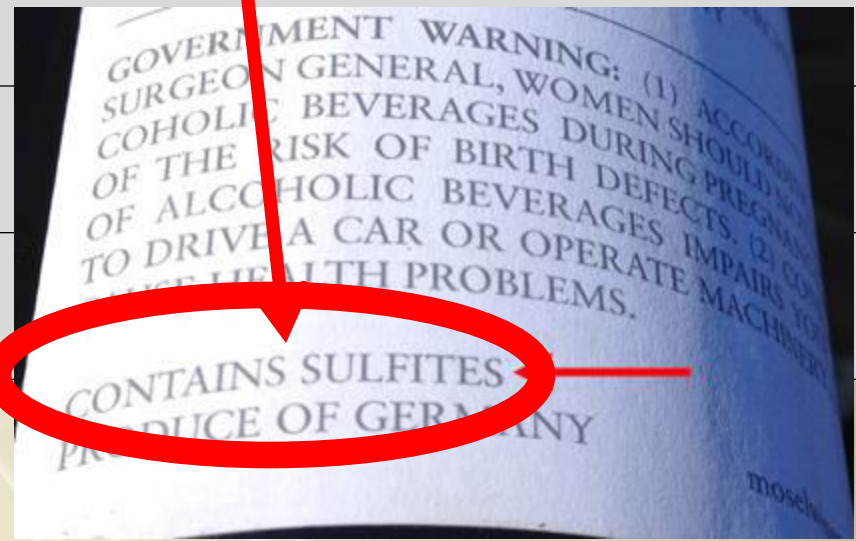
Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
SO ₂	Asthmatics	



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



Use other new preservatives or technologies



Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
SO ₂	Asthmatics	Labeled: It contains sulfites. Other preservatives (sulfites free) Legal limits
Allergenic additives	allergic people (Ex: albumin)	

Problem

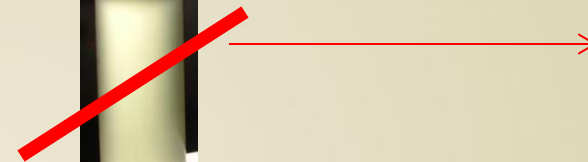
Risk group

Solution (HACCP)

Allergenic additives

allergic people
(Ex: albumin)

Labeled.
Or non allergenic additives



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



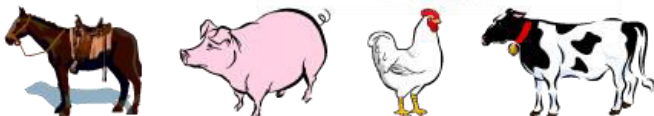
substance obtained from dried fish bladders

Casein

a protein found in cows milk

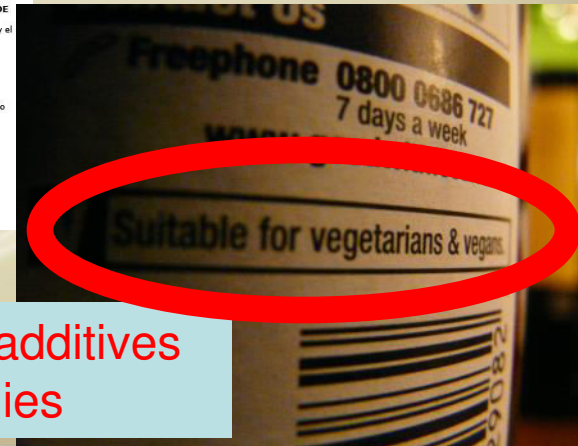


Gelatin



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

himmeeand123.com

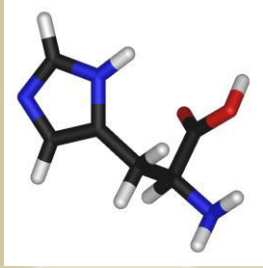


Use of new additives or technologies

Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
SO ₂	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)	

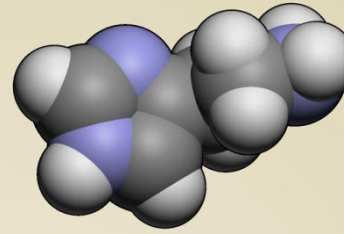
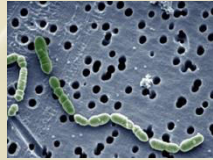
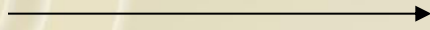
Biogenic Amines Problem

Origin



Histidine

Decarboxylase
enzymes



Histamine

Others:

-Tyramine

-Putrescine

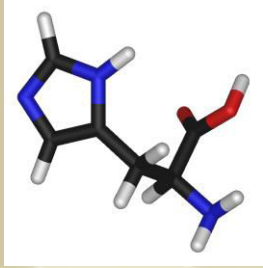
-Cadaverine

-Phenylethylamine

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

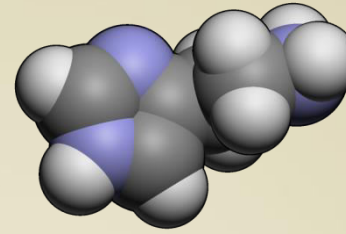
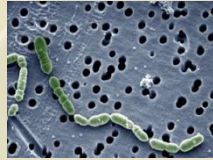
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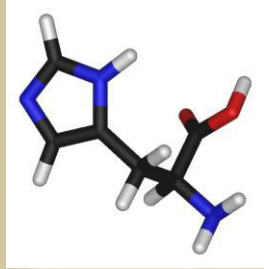
Lactic Bacteria (*O. Oeni*) metabolism => Biogenic Amines ↑

Real Group of Risk

Allergic People => Biogenic Amines + Alcohol

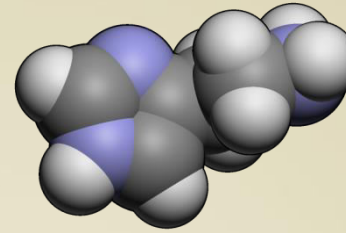
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Lactic Bacteria (*O. Oeni*) metabolism => Biogenic Amines ↑

Real Group of Risk

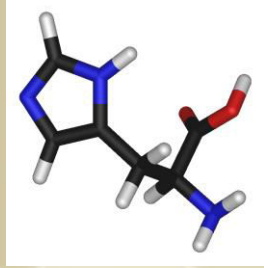
Alergic People => Biogenic Amines + Alcohol

Legal Limits

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

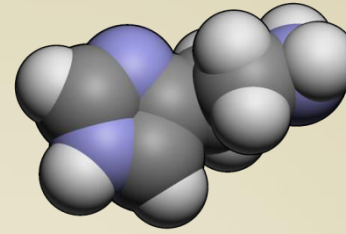
Biogenic Amines Problem

Origin



Histidine

Decarboxylase
enzymes



Histamine

Others:

- Tyramine
- Putrescine
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- Phenylethylamine

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

Real Group of Risk

Alergic People => Biogenic Amines + Alcohol

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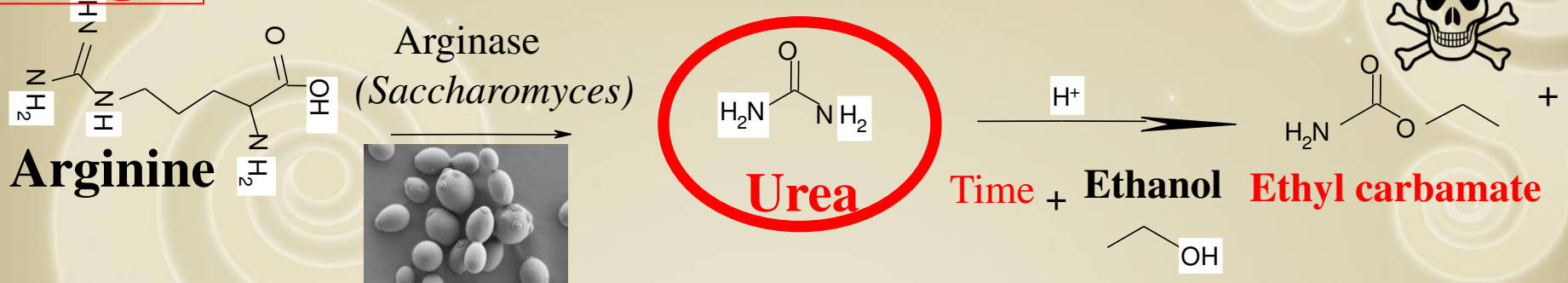
**Germany, Switzerland, Belgium, Austria, Holland, France =>
2-10 mg/L (Histamine)**

How should we control it ?

Problem	Risk group	Solution (HACCP)
Ethanol	Alcoholics, diabetics,	Labeled Free alcohol wine
Physical hazards	Anyone	Filtration
SO ₂	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)	legal limits => but no viable industrial solution ?
Ethyl Carbamate	Anyone (carcinogenic)	

Origin

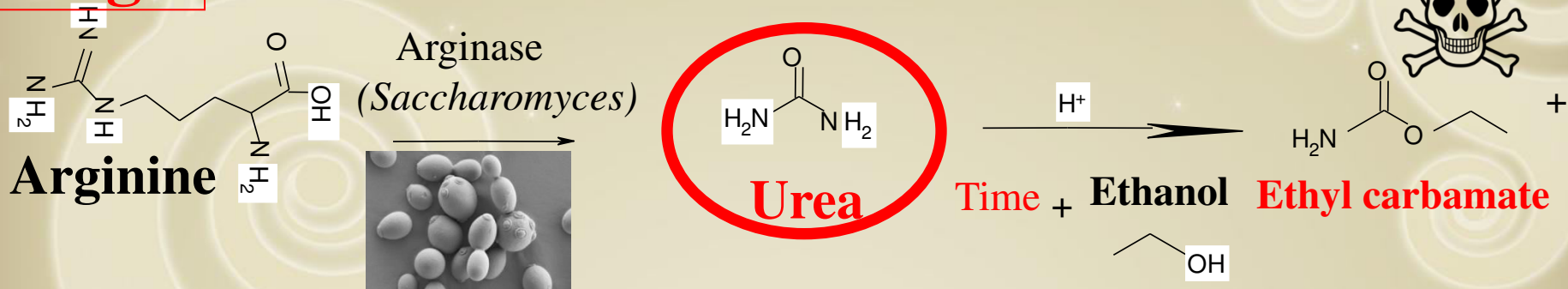
Ethyl Carbamate Problem



Yeast metabolism \Rightarrow Ethyl Carbamate Precursor \uparrow (UREA)
Uthurry et al.2004; Bertrand 1993

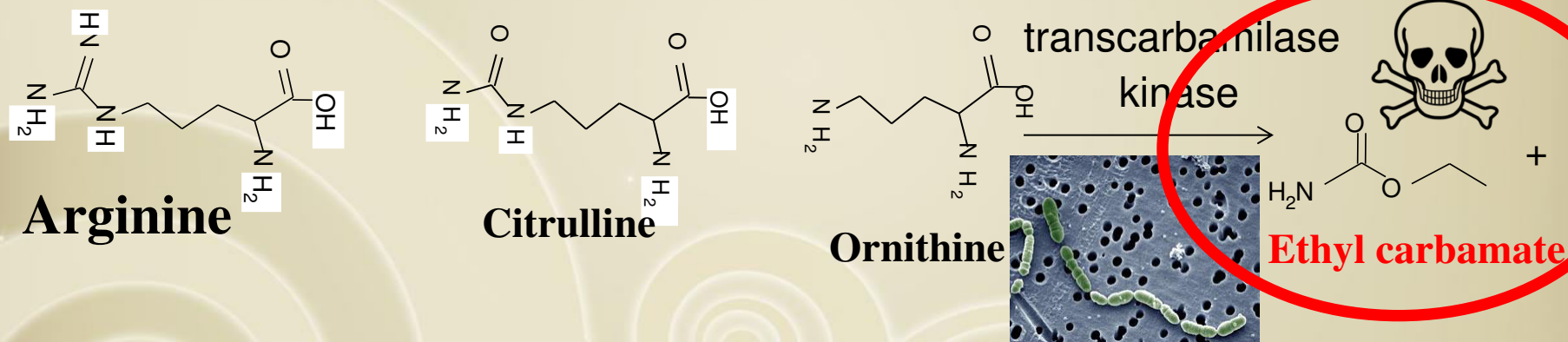
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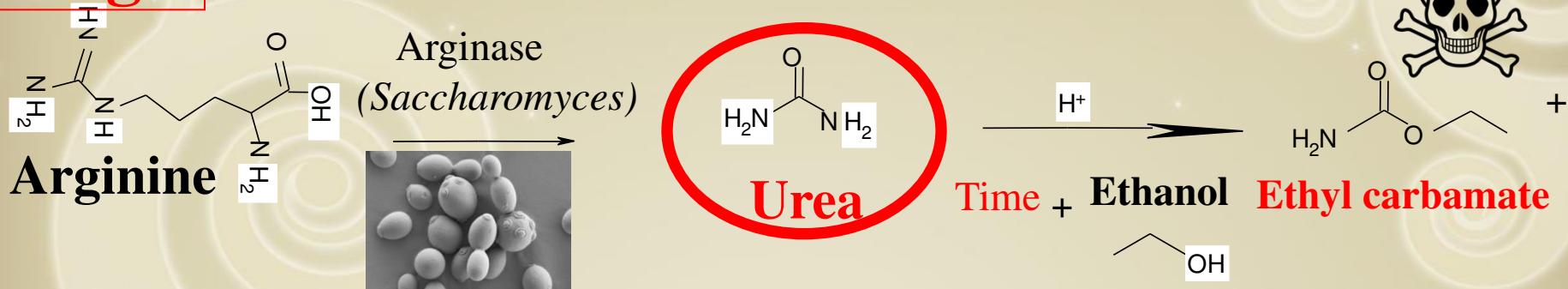


Lactic Bacteria (*O. Oeni*) metabolism \Rightarrow Ethyl Carbamate \uparrow

Tegmo-Larsson et al.1989

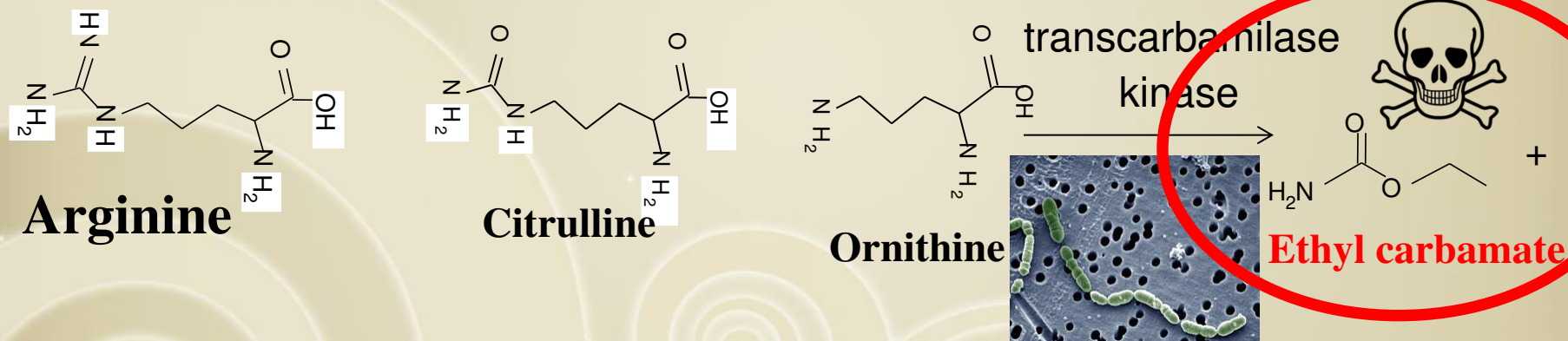
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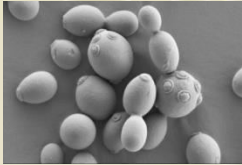
Legal Limits

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

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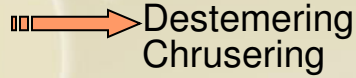
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Biogenic Amines	allergic people (Ex: Histamine)	legal limits => but no viable industrial solution ?
Ethyl Carbamate	Anyone (carcinogenic)	legal limits => but no viable industrial solution ?



PRODUCTION OF RED WINE



Reception



Destemmering
Chrusering



Prefermentative
Maceration



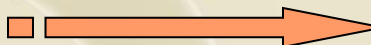
1^o Fermentation
Maceration



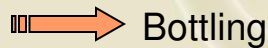
Pressing



2^a Fermentation
Maceration



Filtration.
Stabilization

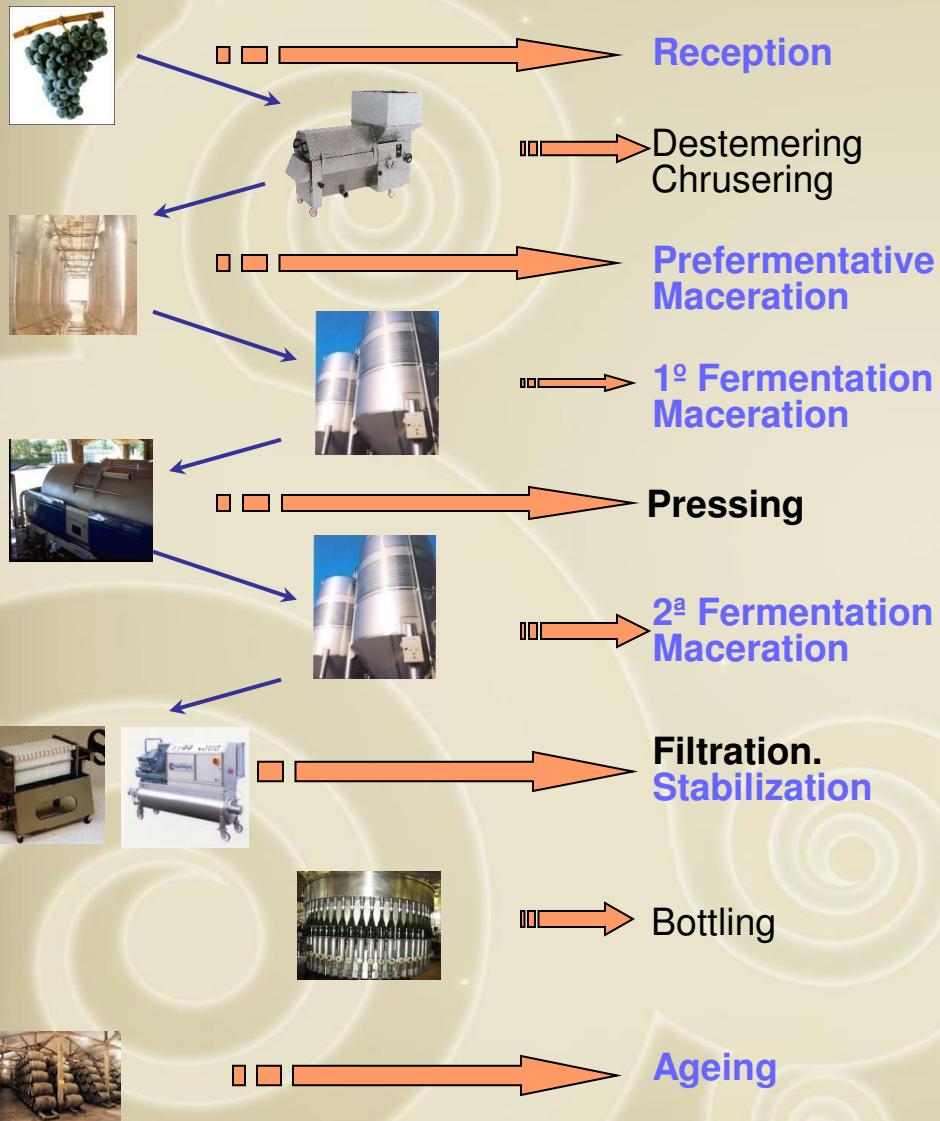


Bottling

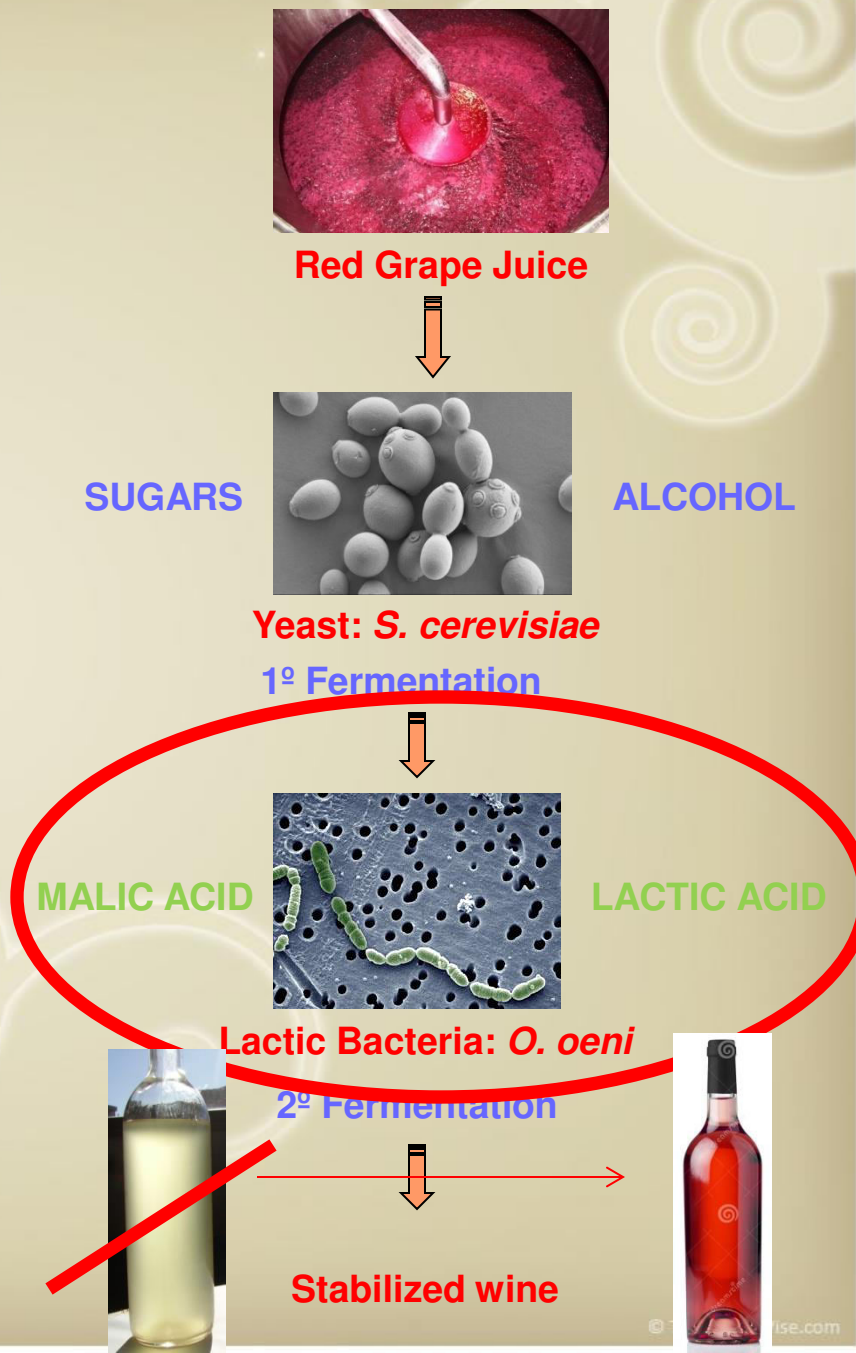


Ageing

PRODUCTION OF RED WINE



MICROBIOLOGICAL SIMPLIFICATION



INDUSTRIAL ALTERNATIVE PROPOSAL



Red Grape Juice



SUGARS
MALIC ACID



ALCOHOL
ETHANOL

Yeast: *Schizo. pombe*
Only 1^o Fermentation



Stabilized wine



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

- Biogenic amines ↓
- Ethyl carbamate ↓

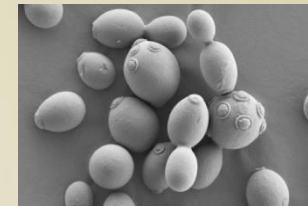
MICROBIOLOGICAL SIMPLIFICATION



Red Grape Juice



SUGARS



ALCOHOL

Yeast: *S. cerevisiae*
1^o Fermentation

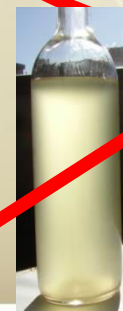


MALIC ACID



LACTIC ACID

Lactic Bacteria: *O. oeni*
2^o Fermentation

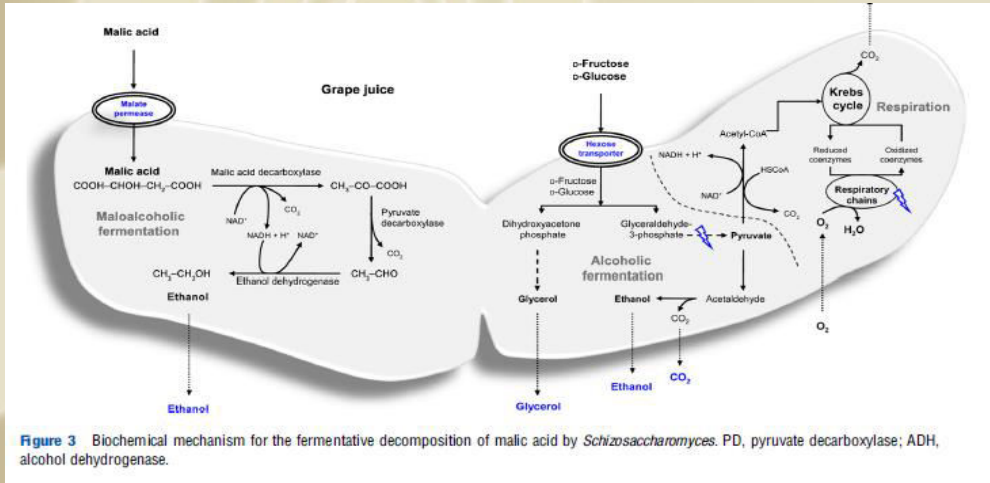


Stabilized wine



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. Déak 2008..

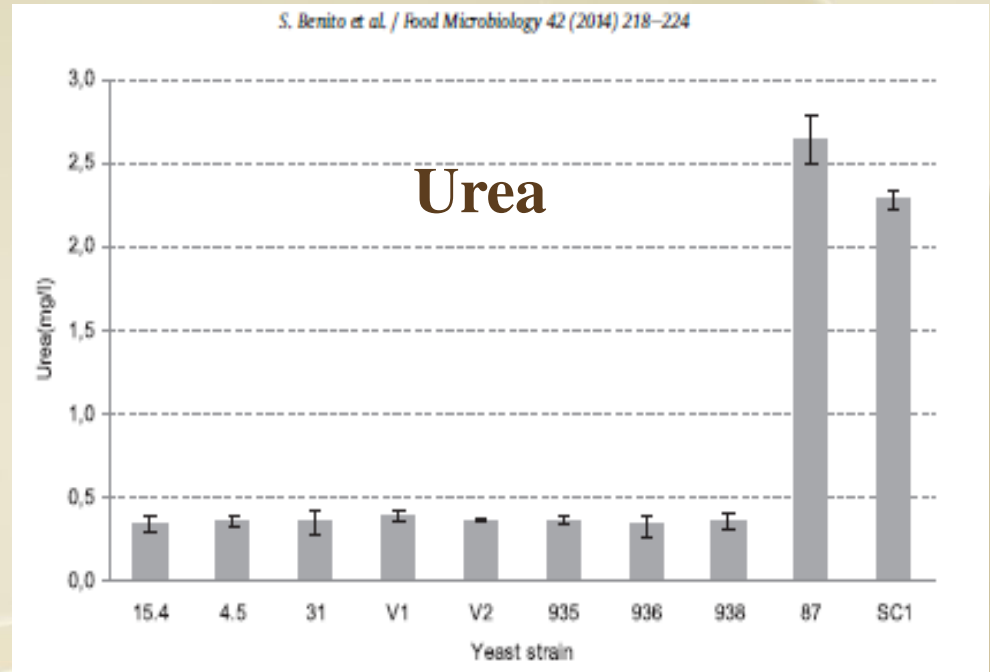
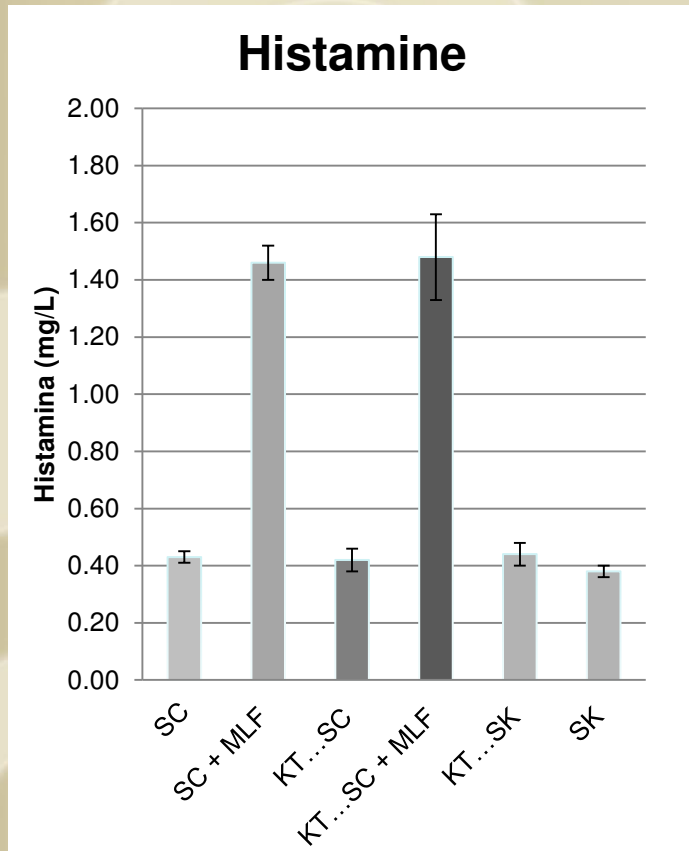
<i>Bulleromyces albus</i>	<i>Filob capsuligenum</i>	<i>Schizo. japonicus</i>
<i>Cry.albidus</i>	<i>F'ella neoformans</i>	<i>Schizo. octosporus</i>
<i>Cry. curvatus</i>	<i>Leucosp scotti</i>	<i>Schizo. pombe</i>
<i>Cry. diffyuens</i>	<i>Moniliella suaveolens</i>	<i>Spori. pararoseus</i>
<i>Cry. humicolus</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*.

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

Bibliography

- Benito S, Palomero P, Morata A, Calderón F, Suárez-Lépe JA (2012) New applications for *Schizosaccharomyces pombe* in the alcoholic fermentation of red wines. *Int J Food Sci Tech* 47:2101-2108
- Benito S, Palomero F, Morata A, Calderon F, Palmero D, Suárez-Lepe JA (2013) Physiological features of *Schizosaccharomyces pombe* of interest in making of white wines. *Eur Food Res Technol* 236:29-36
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- Benito S, Palomero P, Calderón F, Palmero D, Suárez-Lepe JA (2014b) *Schizosaccharomyces*. In: Batt CA, Tortorello ML (eds) *Encyclopedia of Food Microbiology*, vol 3, 2nd edn. Elsevier Ltd, Academic Press, Amsterdam, pp 365-370
- Benito S, Palomero P, Gálvez L, Morata A, Calderón F, Palmero D, Suárez-Lepe JA (2014c) Quality and Composition of Red Wine Fermented with *Schizosaccharomyces pombe* as Sole Fermentative Yeast, and in Mixed and Sequential Fermentations with *Saccharomyces cerevisiae*. *Food Technol Biotechnol* 52:376-382
- 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