REDUCING SULPHITES CONTENT IN WINES

REDUCING
SULPHITE CONTENT

LOw SO_2
SOLUTIONS

Bioprotection,
Vinification, Storage

IOC
Révélons votre différence

Consumers and sulphites in wine

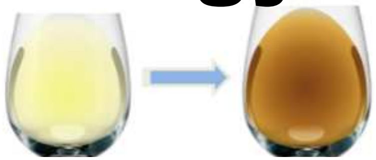
A cause du vin, un million de Français en surdose de sulfites

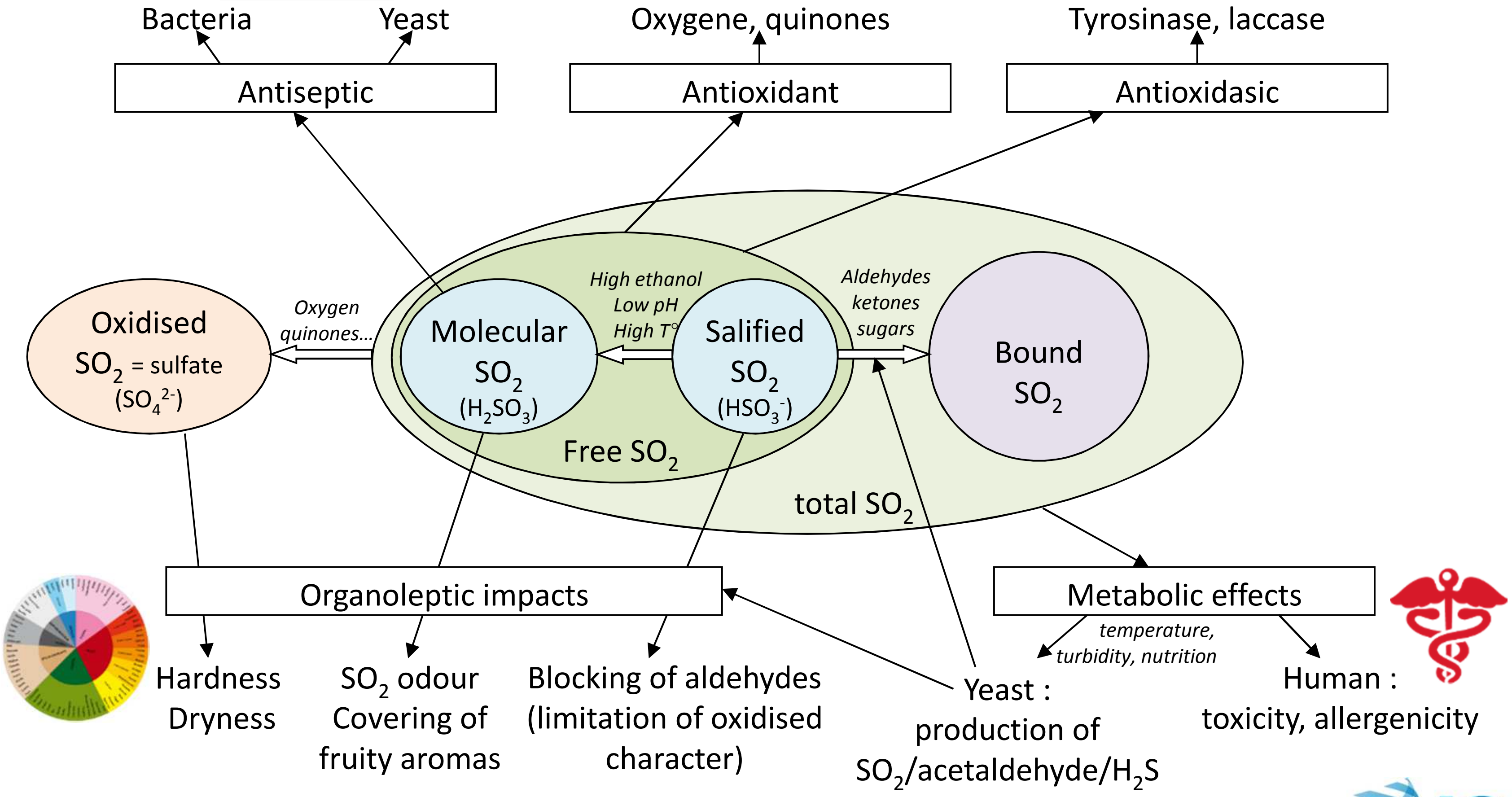
Par Antonin Iommi-Amunategui. Publié le 27/08/2012 à 10h31

172 103 VISITES 203 RÉACTIONS ● 11

Des verres de vin blanc - Eflon/Flickr/CC

Roles and impacts of SO_2 in Oenology





Bacteria

Yeast

Oxygene, quinones

Tyrosinase, laccase

Antiseptic

Antioxidant

Antioxidasic

Oxidised SO_2 = sulfate (SO_4^{2-})

Oxygen quinones…

Molecular SO_2 (H_2SO_3)

High ethanol
Low pH
High T°

Salified SO_2 (HSO_3^-)

Aldehydes
ketones
sugars

Bound SO_2

Free SO_2

total SO_2

Organoleptic impacts

Metabolic effects

temperature, turbidity, nutrition

Hardness
Dryness

SO_2 odour
Covering of fruity aromas

Blocking of aldehydes (limitation of oxidised character)

Yeast :
production of SO_2/acetaldehyde/H_2S

Human :
toxicity, allergenicity

> ***« Diversity is the place of art »***
>
> *Albert Camus*

Microbiological cartography of grape must

DIVERSITY OF FLORAS:
RISKS AND BENEFITS

Which flora on grapes ?

Vintage 2013

Hand harvest *(nearly 20 kgs)*

Healthy grapes from “organic” parcels

(Pinot noir x2, Chardonnay and Sauvignon)

Direct pressing

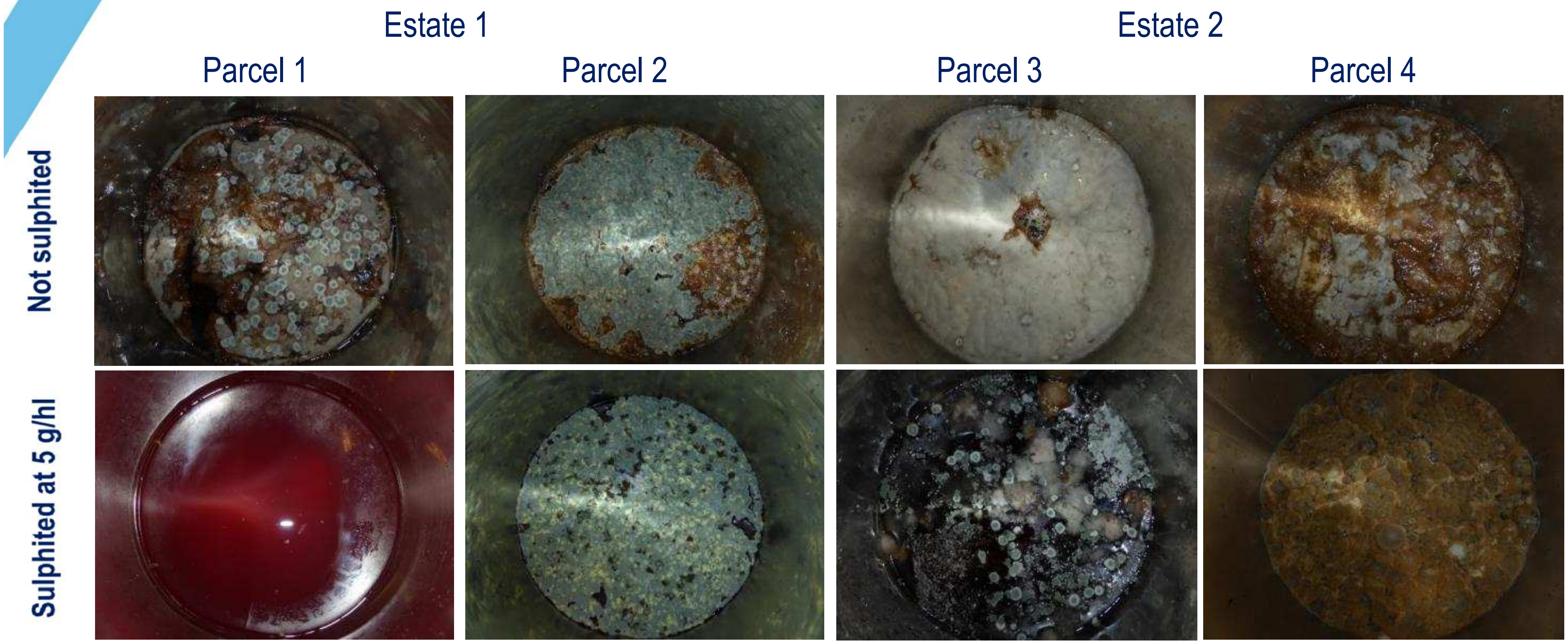
Divided in 2 deposits of 15 L

Addition of sulphite 5 g/hL or none

Every operation carried out with sterile equipment .

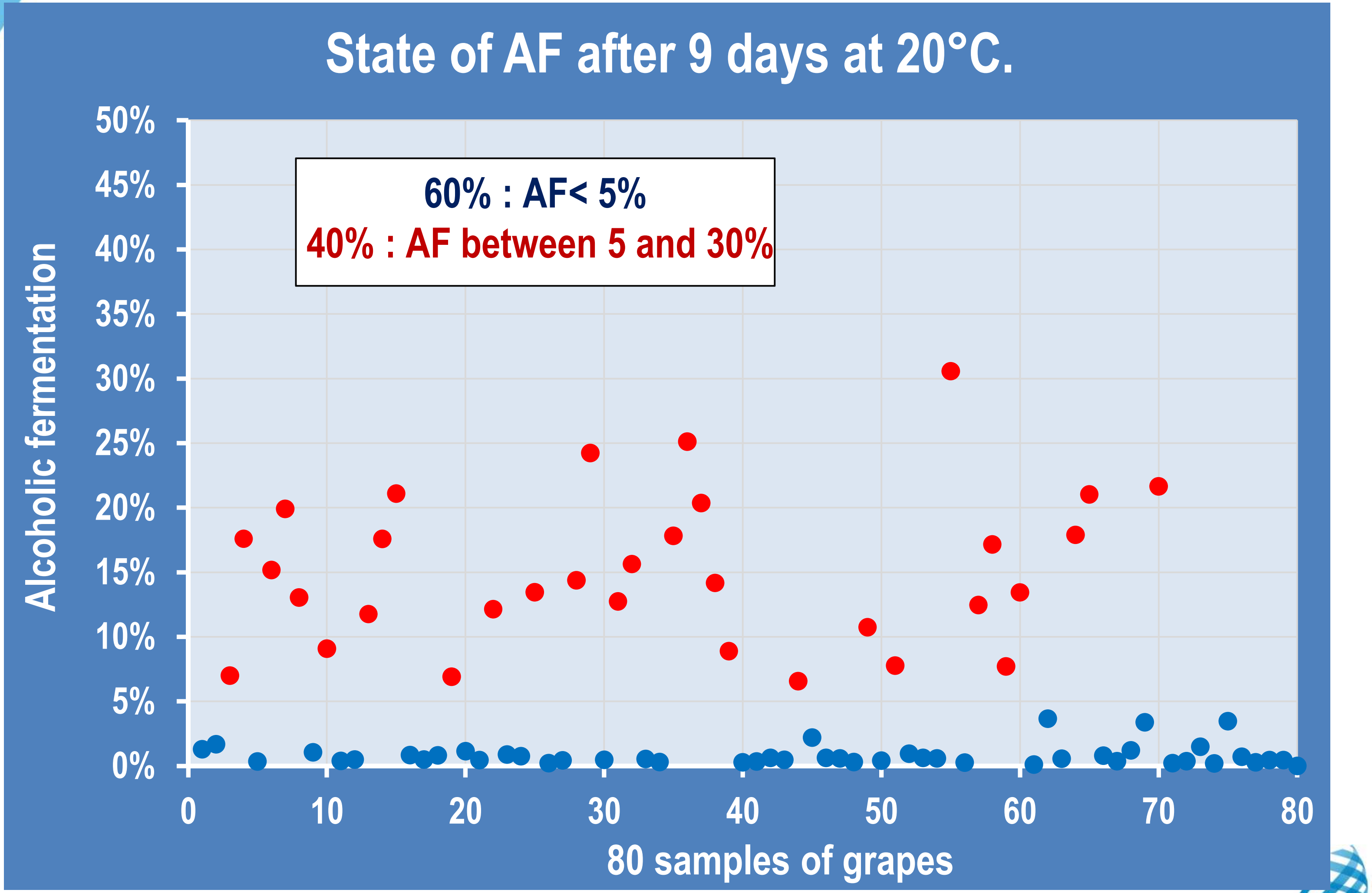
Which flora on grapes ?

Photographs of surface after 9 days at 20°C.

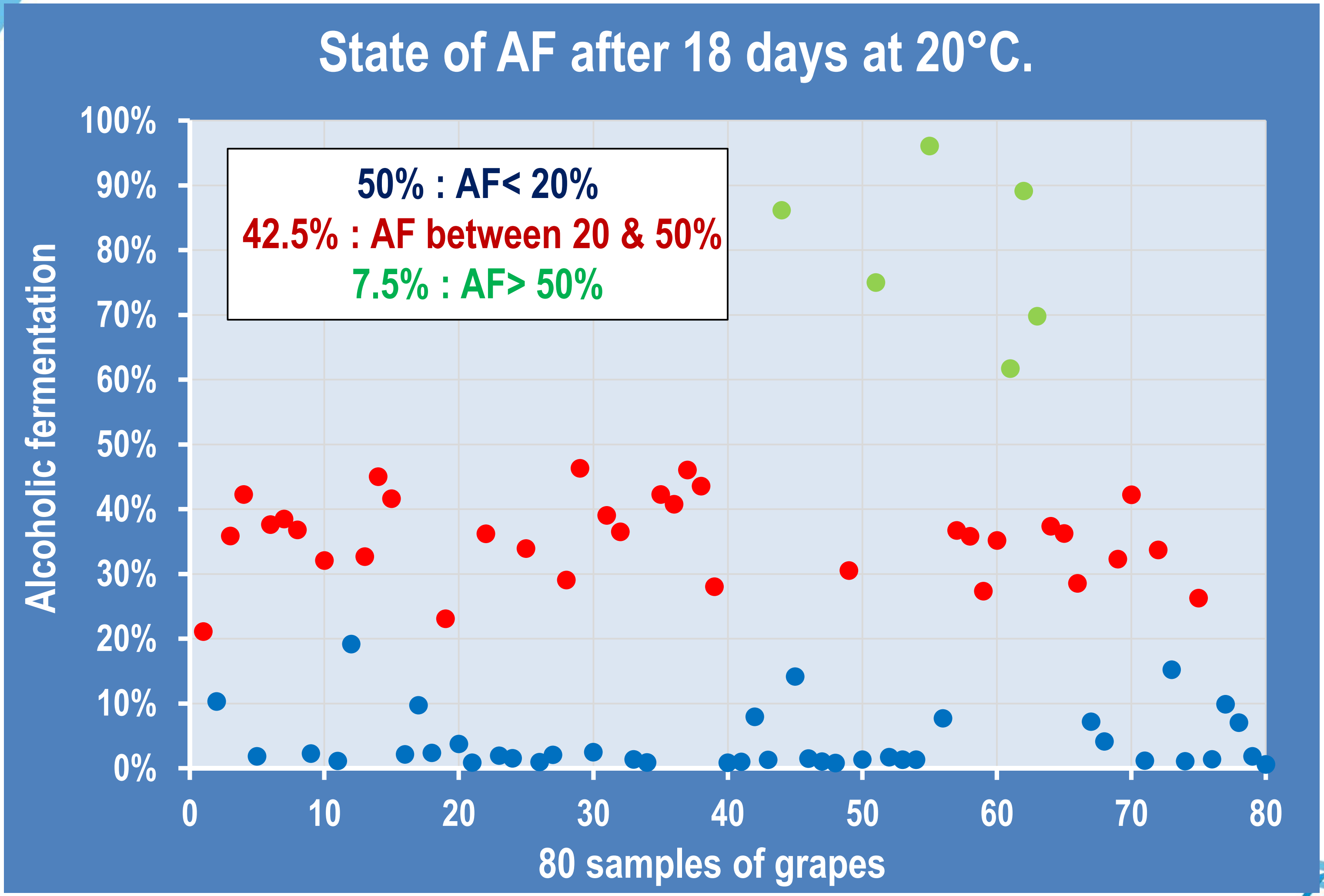


Molds is growing quickly in surface.
AF hasn't triggered.

Which flora on grapes ?



Which flora on grapes ?



Which flora on grapes ?

Micro-organisms on (healthy) grapes

Grapes are contaminated by mold.

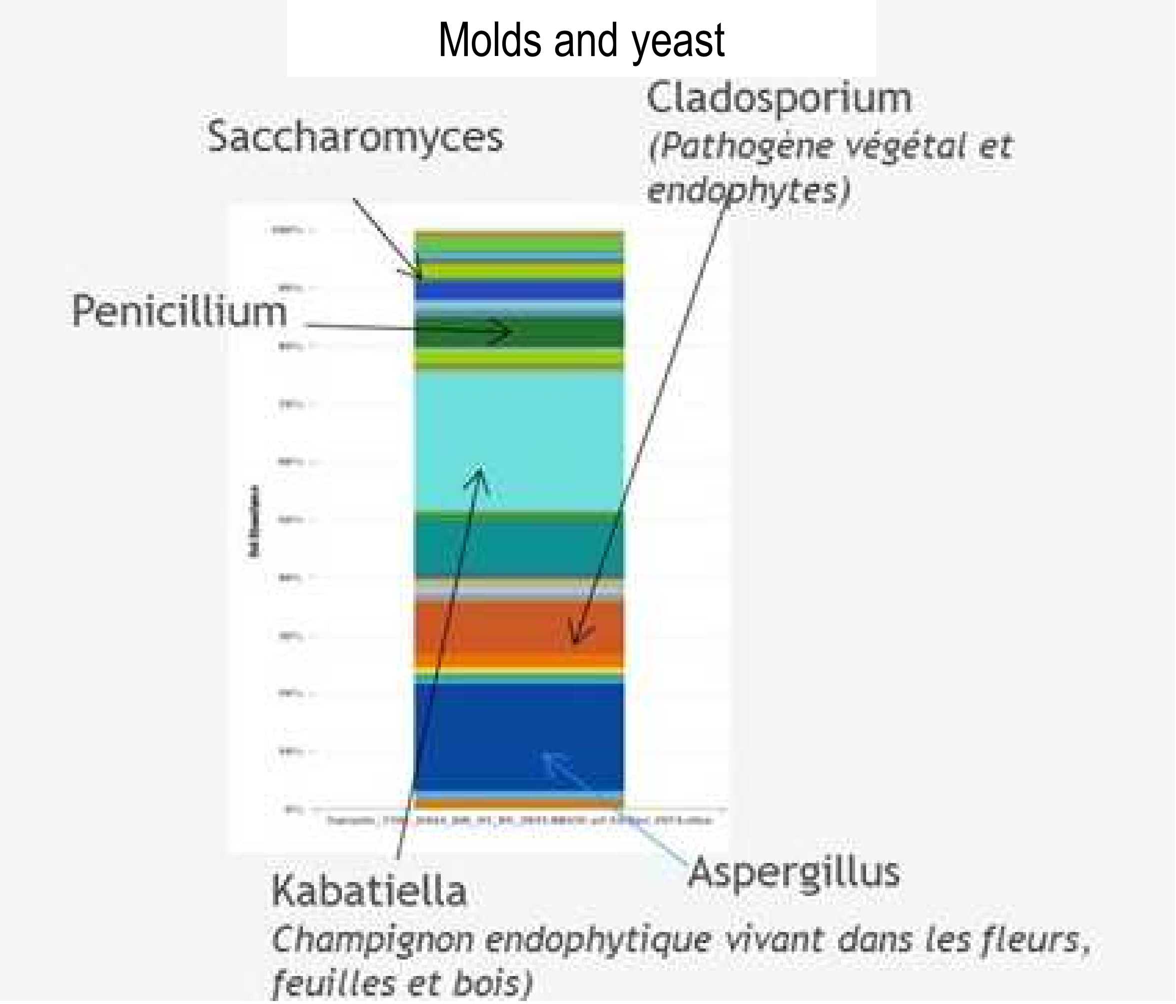
Grapes are contaminated by yeasts :

- Presence of yeast with low fermentative power and high potential of acetic acid production (such as *Hanseniaspora*).
- Presence of yeast with very low fermentative power and with very low potential of acetic acide production (such as *Metschnikowia*).

⇨ - Very low presence of yeast with strong fermentative power (such as *Saccharomyces*).

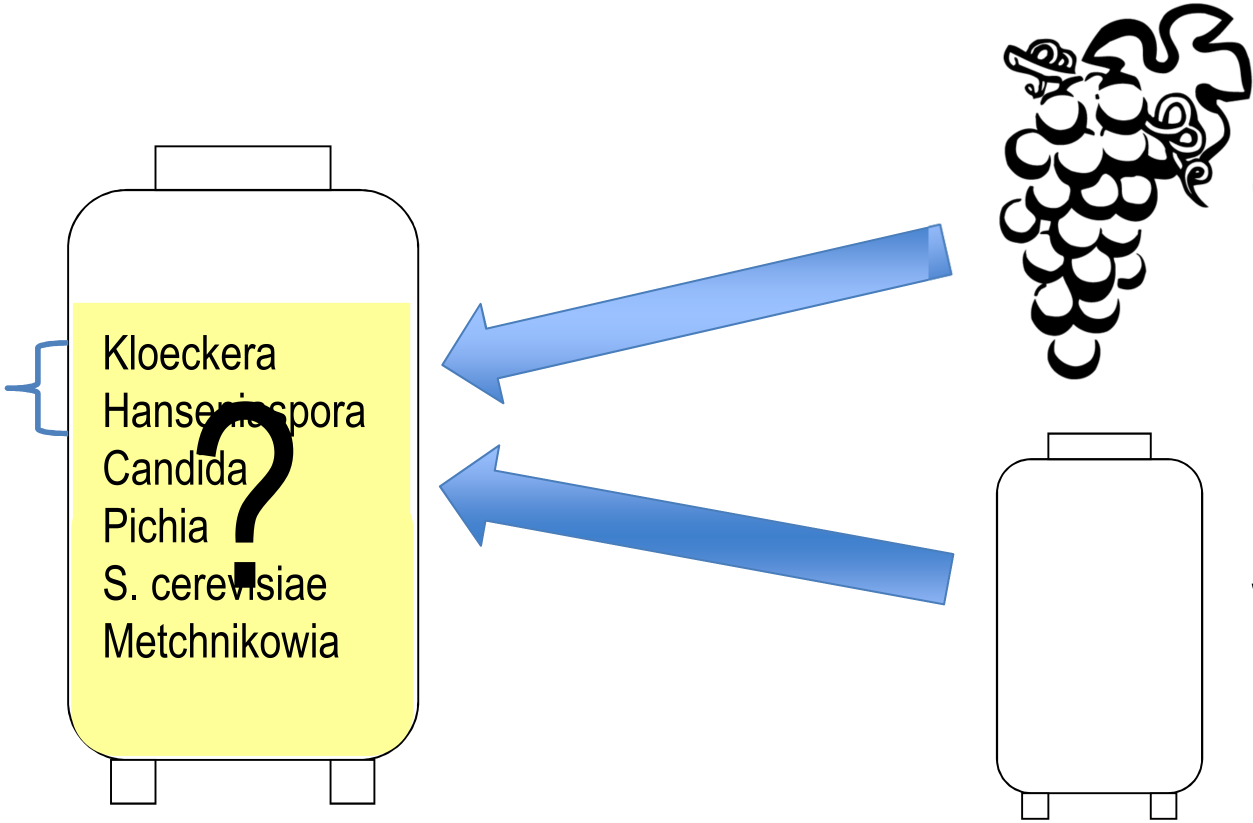
Diversity of micro-organisms on grape

Molds and yeast



Metagenomic survey on merlot grapes (according to Bauquis, 2017)

Yeast diversity in must : a double component



Often majority (>70%)

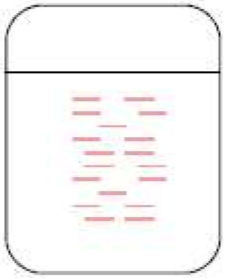
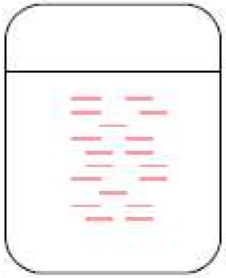
Kloeckera
Hanseniaspora
Candida
Pichia
S. cerevisiae
Metchnikowia

Grape flora at maturity

Flora present on winery equipment:
Saccharomyces
Candida
Brettanomyces

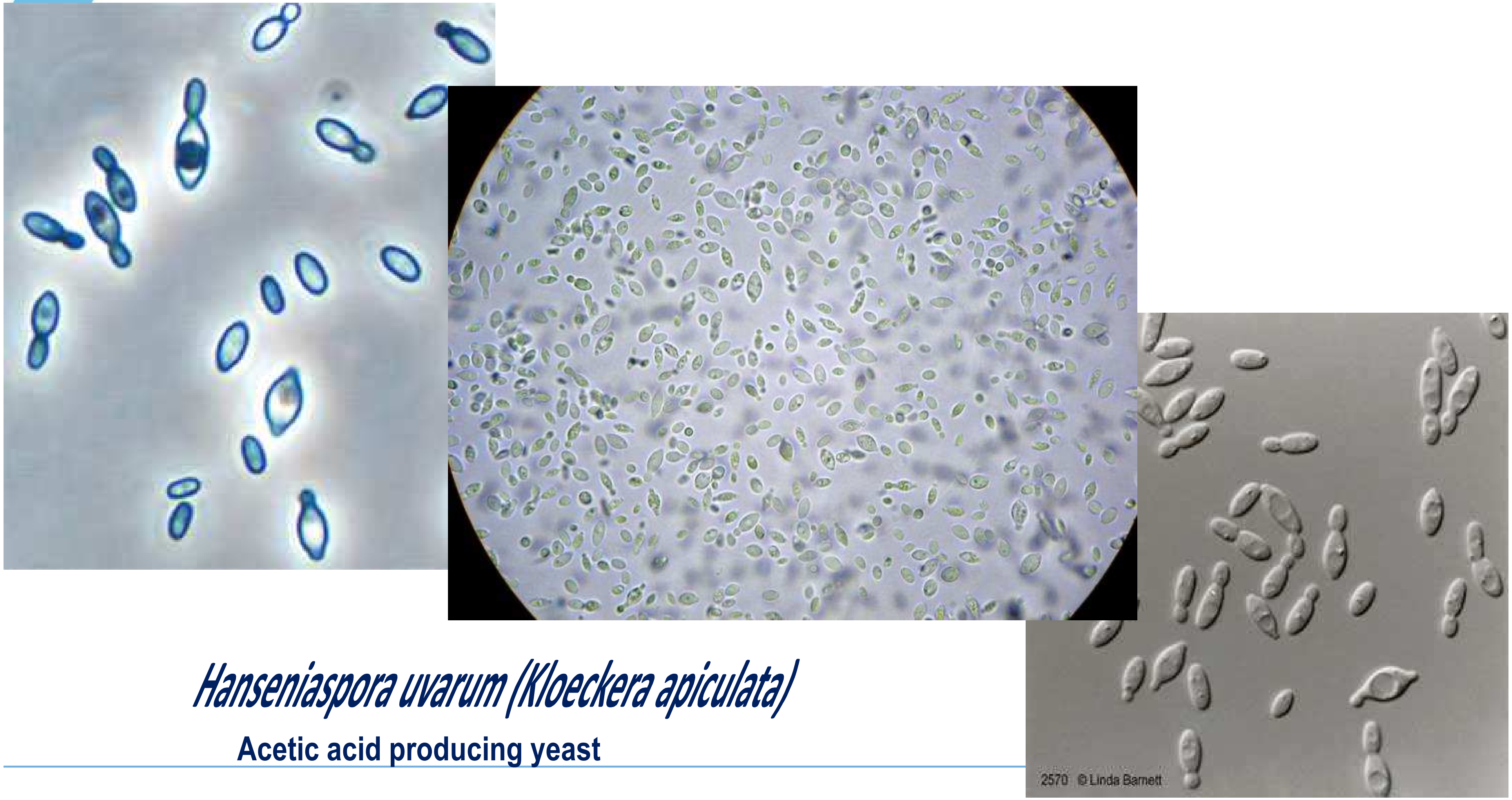
Variable levels of population:
10^3 to 10^6 cell/ mL

Evolution of yeasts floras

Vine	Must, beginning of AF	Wine during AF >6% alcohol	End of AF, wines
			
Kloeckera *Hanseniaspora* *Rhodotorula* *Candida* *Metchnikowia*	*Kloeckera* *Hanseniaspora* *Candida* *S. cerevisiae* *Metchnikowia*	*S. cerevisiae*	*S. cerevisiae* *Zygosaccharomyces* *Brettanomyces* Resistant to SO_2 and alcohol

According to Blondin, 18 mars 2011, matinée technique des œnologues (Montpellier SupAgro)

The specific case of cold soak



Hanseniaspora uvarum (Kloeckera apiculata)

Acetic acid producing yeast

The specific case of cold soak

Growth of *Hanseniaspora uvarum* in must.

Must of pasteurized Pinot noir : sugars 230 g/l, pH 3.20, no SO2

Incubation at 15°C

Yeast (cell./ml)	**T0**	**T 1 day**	**T 6 days**
Control (non contaminated)	< 10	< 10	< 10
Hanseniaspora uvarum (contaminated)	**320**	**22 000**	**70 000 000**

The specific case of cold soak

Activity of *Hanseniaspora uvarum* in must.

Must of pasteurized Pinot noir : sugars 230 g/l, pH 3.20, no SO2

Cold soak at 15°C – Yeast addition (Sac.c.) at T7 days – AF at 20 / 24°C.

Acetic acid (g/l)	**End cold soak** (T 7 days)	**End AF** (T 14 days)
Control	0.02	0.35
***Hanseniaspora uvarum* ***	**0.31**	**0.67**

*** *Hanseniaspora* produces nearly 10 times more ethylacetate than *Saccharomyces.***

Impact of temperature

- Low temperatures can promote *non-Saccharomyces* (but also *Saccharomyces uvarum*).
- At low temperature (15°C), apiculated yeast resist better to alcohol. There are cases of dominance of apiculated yeast at the end of AF !
- To reason together with the level of SO_2.
- On the opposite, high temperatures (28°C) promote *S. cerevisiae.*

According to Blondin, 18 mars 2011, matinée technique des œnologues (Montpellier SupAgro)

Yeast diversity in fermentation: benefits

- Potential production of metabolites of interest:
  - Specific fruity esters
  - Varietal thiols
  - Other aromas…
  - Glycerol

Aromatic complexity

- Specific fermentative capacities of some non *Saccharomyces* strains (osmotolerance, cryophily…)

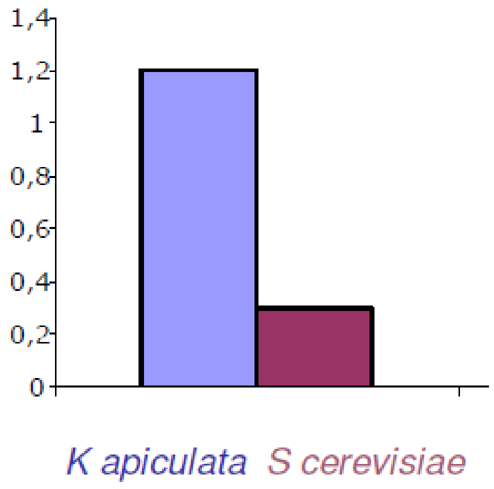
Yeast diversity in fermentation: dangers

- **Potentially high production of acetic acid and ethylacetate.**
- Potentially high production of H2S (linked to the nutrition).

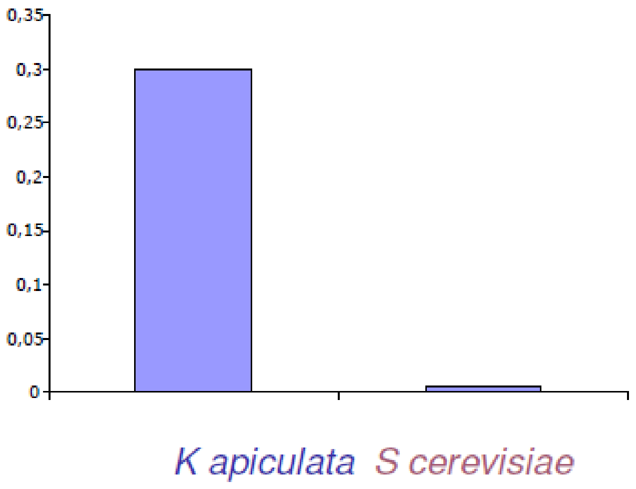
Potential of acetate production

Formation of volatiles during AF

Volatile acidity



Ethylacetate



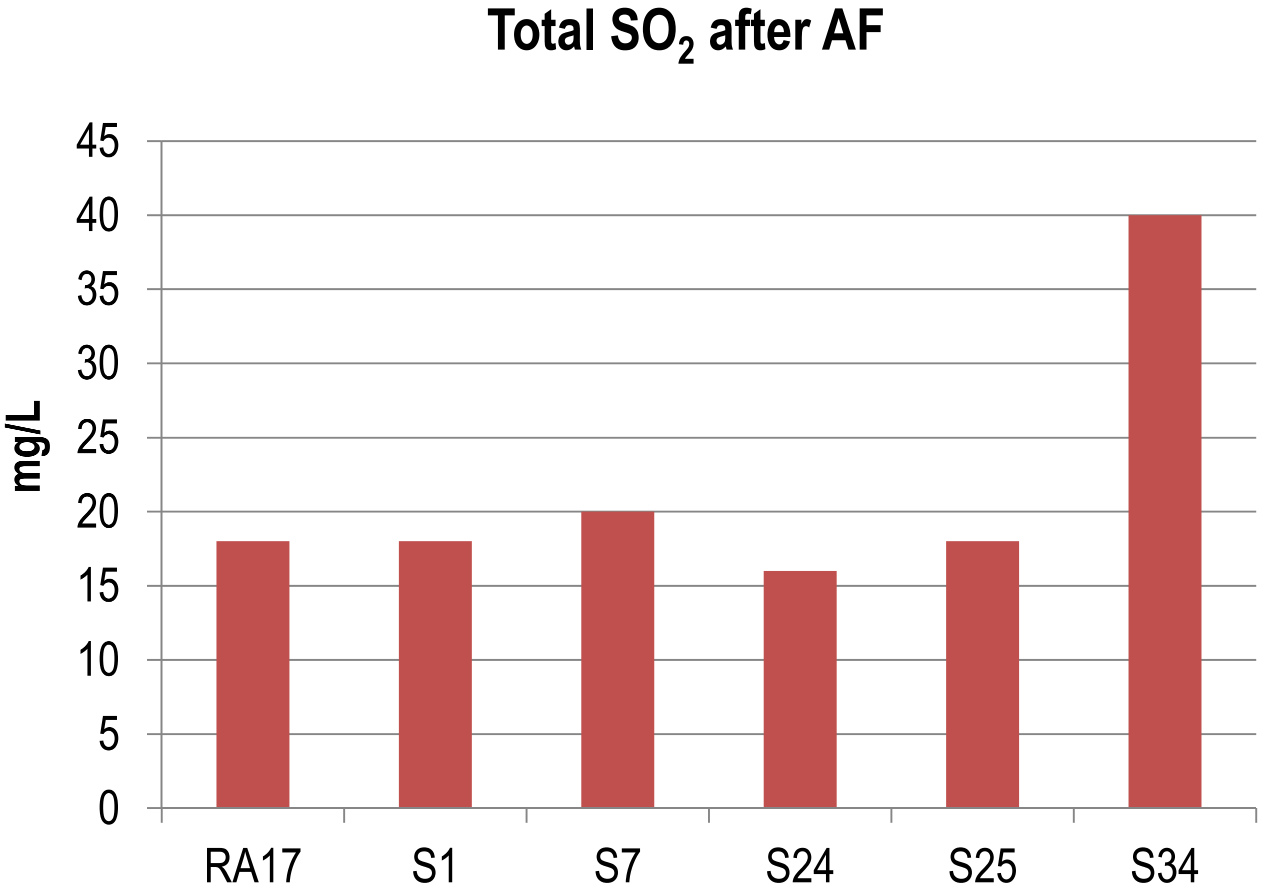
Alcohol reached: S cerevisiae 11,1 °A
K apiculata 6,4 °A

According to Blondin, 18 mars 2011, matinée technique des œnologues (Montpellier SupAgro)

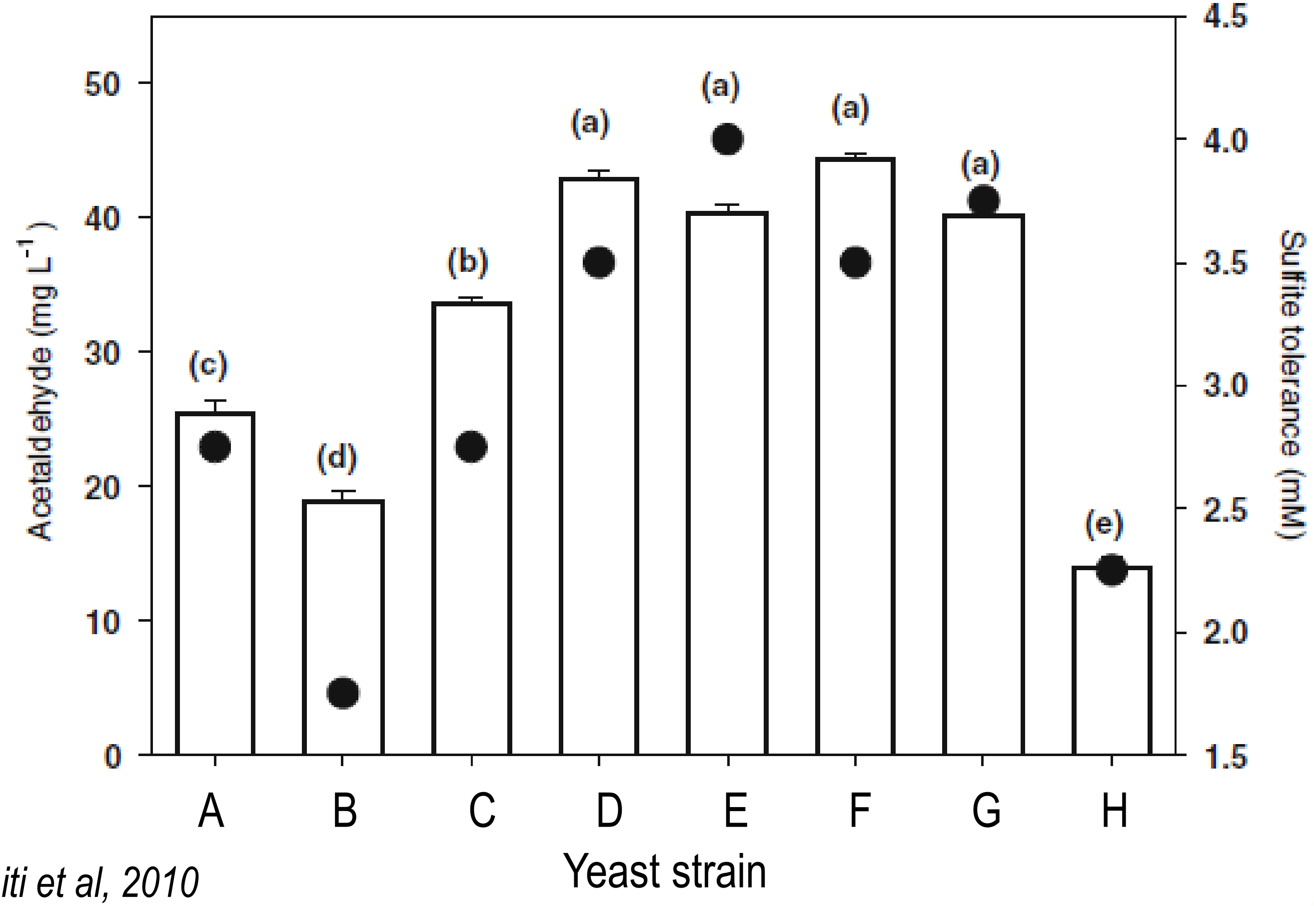
Yeast diversity in fermentation: dangers

- Potentially high production of acetic acid and ethylacetate
- Potentially high production of SO_2 and/or acetaldehyde

Potential of production of SO_2 by yeast



Variability of production of acetaldehyde



According to Cheraiti et al, 2010

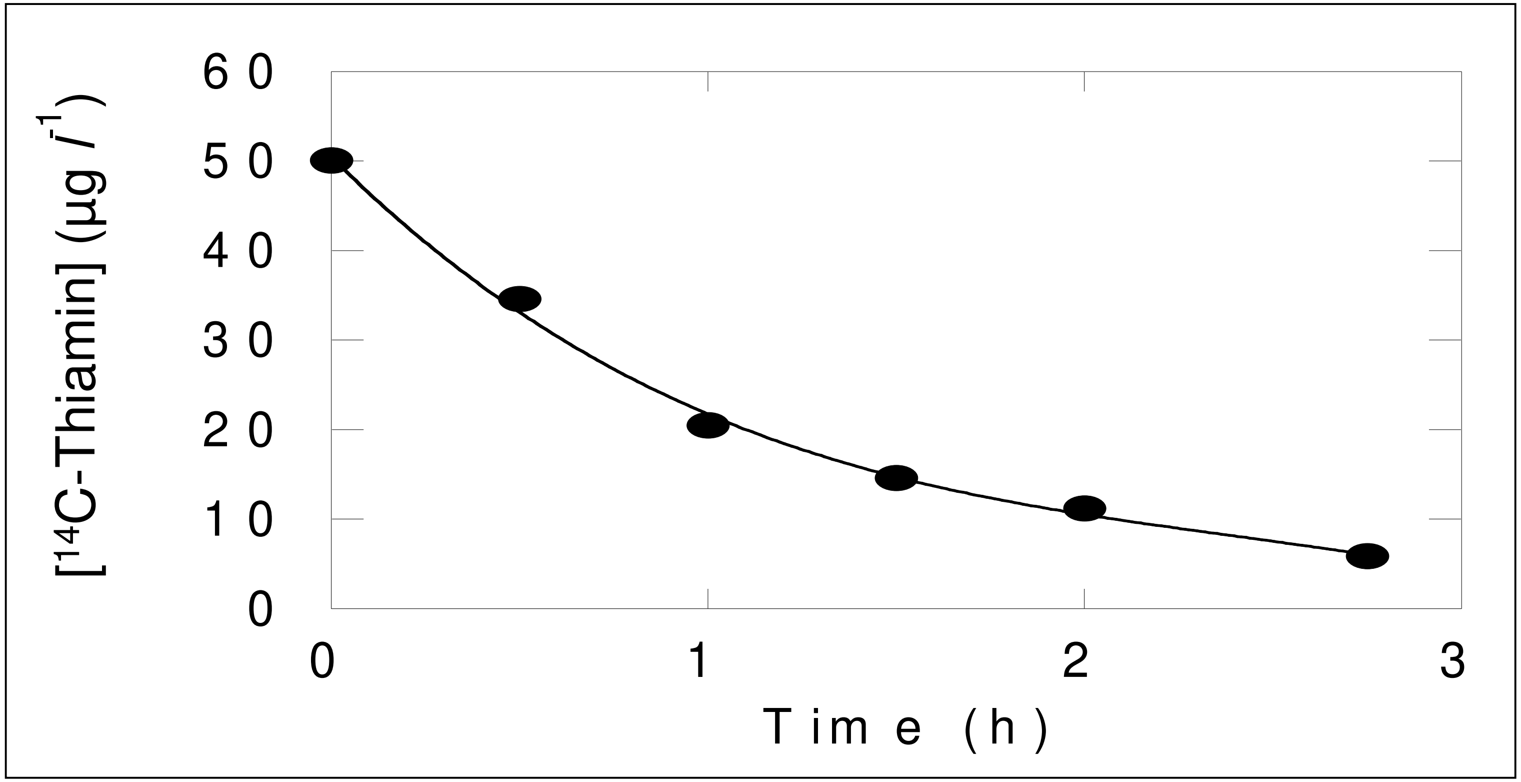
Yeast diversity in fermentation: dangers

- Potentially high production of acetic acid and ethylacetate
- Potentially high production of SO_2 and/or acetaldehyde
- Possible production of volatile phenols (*Brettanomyces bruxellensis, Pichia guillermondi*)

Yeast diversity in fermentation: dangers

- Potentially high production of acetic acid and ethylacetate
- Potentially high production of SO_2 and/or acetaldehyde
- Possible production of volatile phenols (*Brettanomyces bruxellensis, Pichia guillermondi*)
- Negative interactions with *S. cerevisiae*

Consommation de la thiamine par *K. apiculata*



And bacteria ?

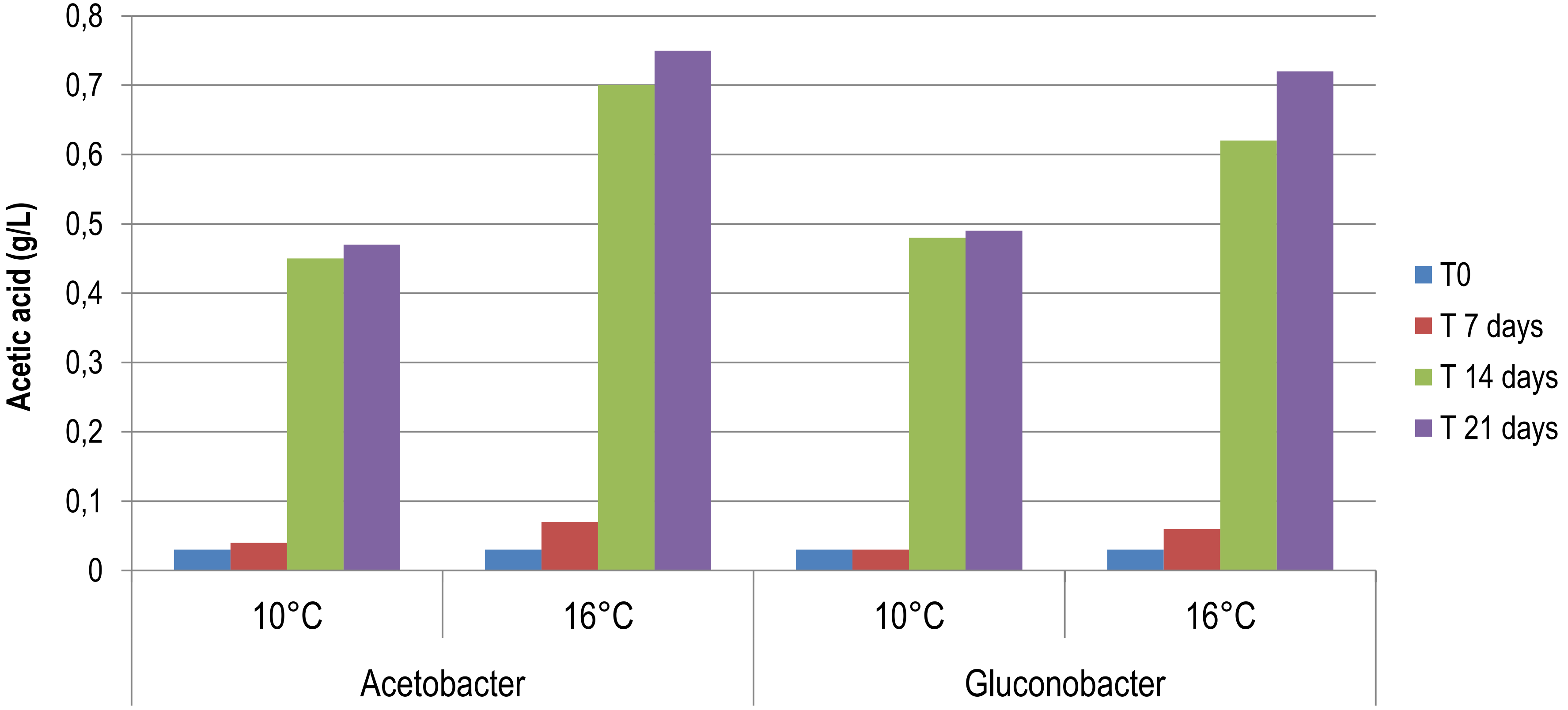
- Sometimes, stronger contaminations

Drosophila suzukii.

And bacteria ?

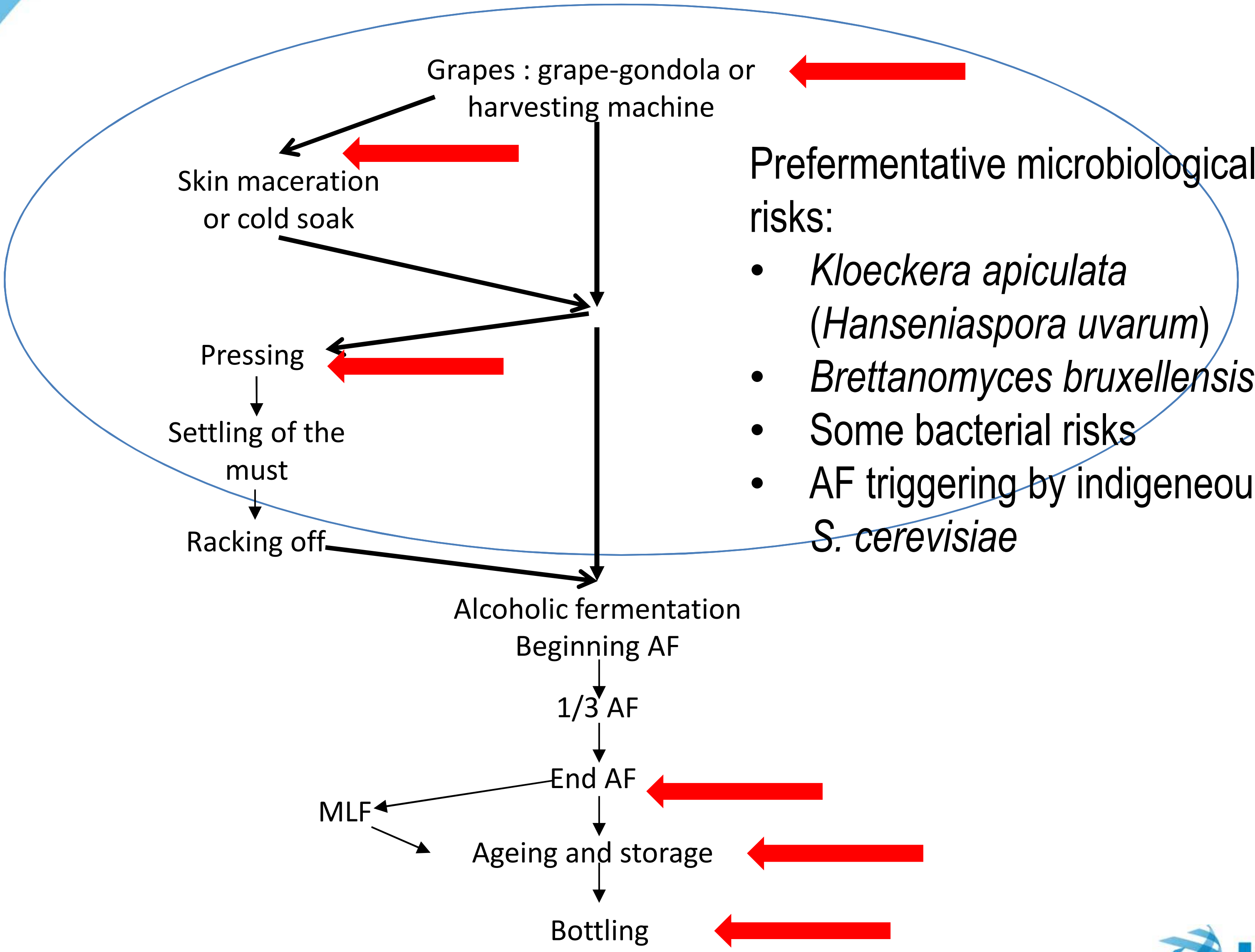
Production of acetic acid after contamination of the must with acetic bacteria

Pasteurized must of pinot noir - inoculation at T0 (104 cell/mL) - prefermentative cold soak at 10°C or 16°C during 7 days then inoculation in S. cerevisiae yeast



Fermentation with local flora : balance benefits/risks

Microbiological risks and sulphiting



Prefermentative microbiological risks:

- *Kloeckera apiculata* (*Hanseniaspora uvarum*)
- *Brettanomyces bruxellensis*
- Some bacterial risks
- AF triggering by indigeneous *S. cerevisiae*

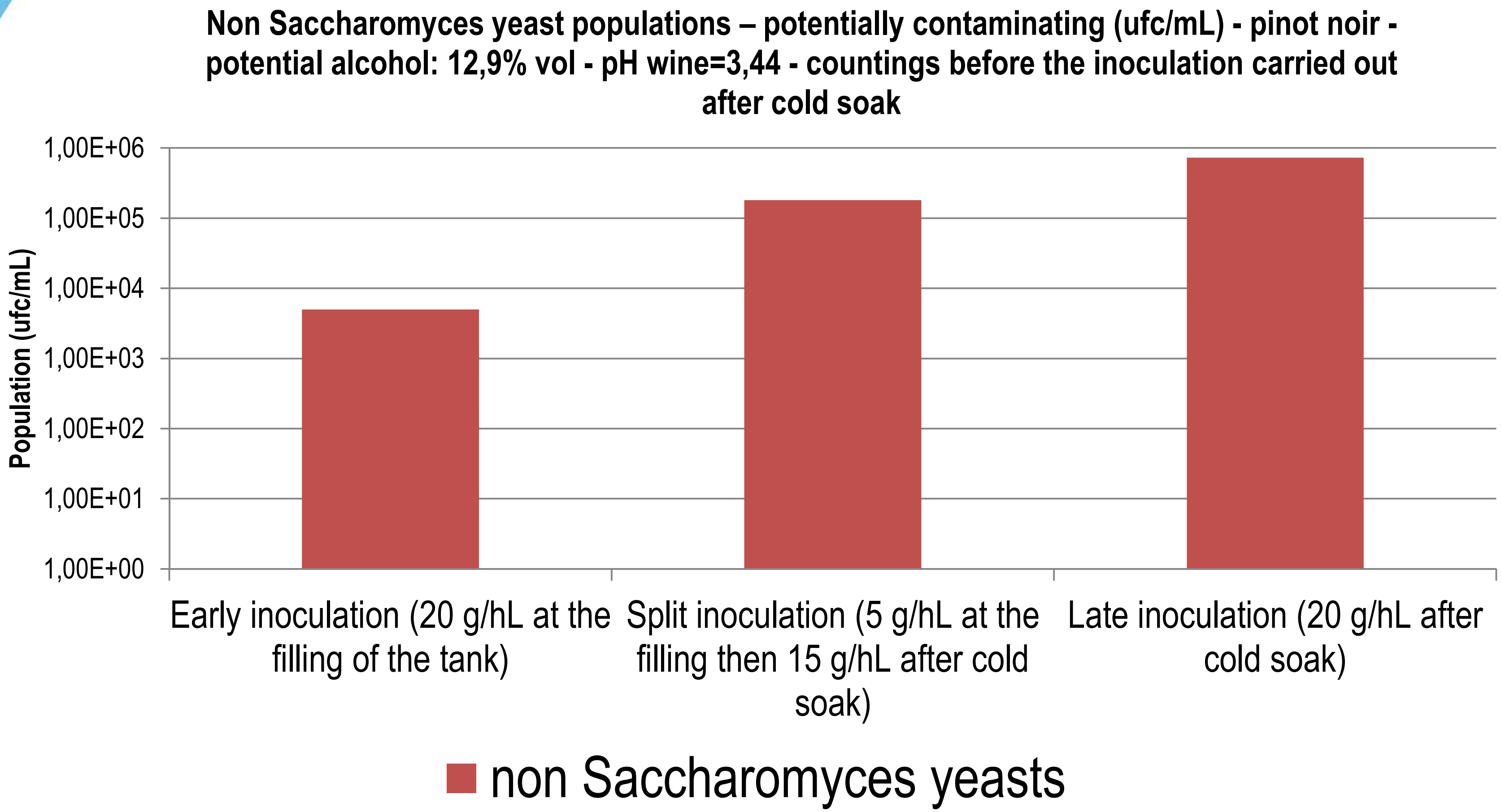
Gaïa™

Metschnikowia fructicola Gaïa™

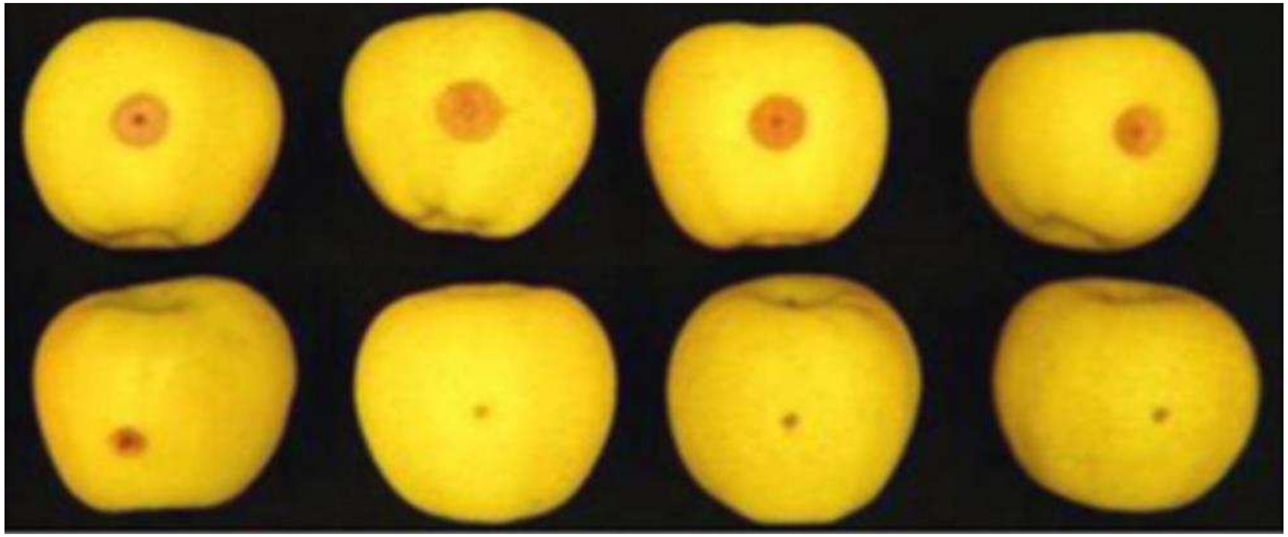
PRE-FERMENTATIVE BIOCONTROL

LOw SO_2 SOLUTIONS

A first approach:
split addition of yeast



M. fructicola and biocontrol : an old story?

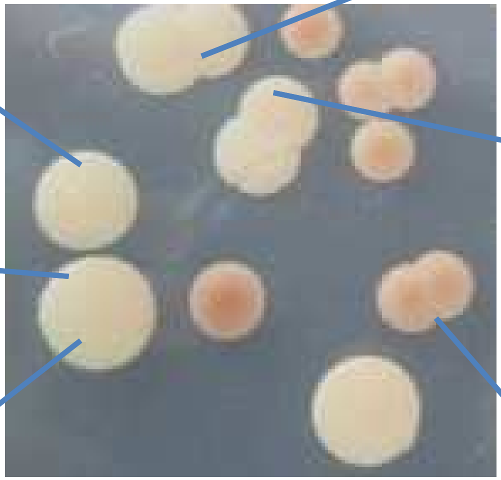


Wound then inoculated apples :
- 1st series with sterile water (10µL)
- 2nd series with a suspension of *M. fructicola* (5.10^7 cells/mL)

2 hours after: inoculation of both series with *Penicilium expansum* then kept at 25°C during 4 days.

Liu et al, 2011 in FEMS Microbiology Ecology

Metschnikowia fructicola Gaïa™



No fermentative power

Low nutritional needs

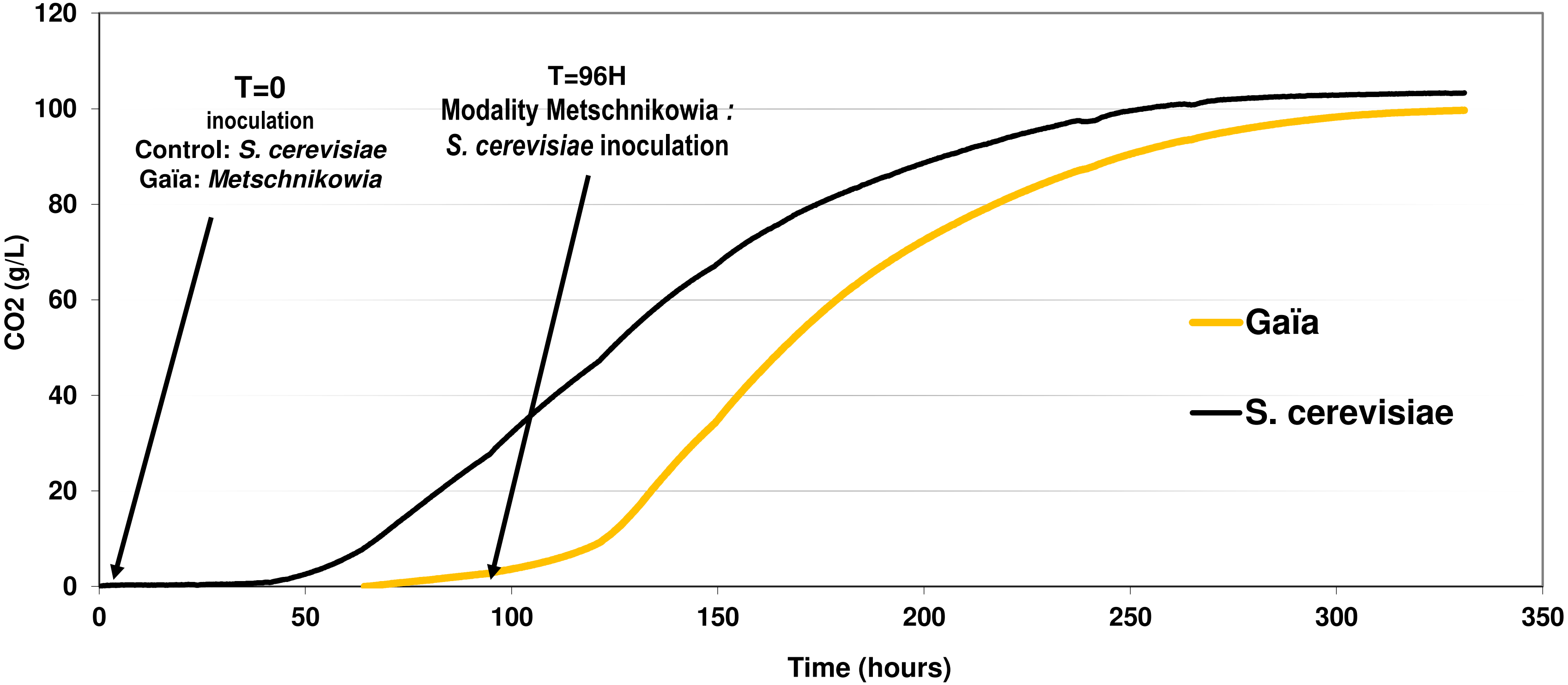
Antimicrobial activity, especially anti-*Kloeckera*

Excellent implantation… and survival

Positive sensory contribution

No production of undesirable metabolites

M. fructicola (Gaïa™): A true prefermentative tool



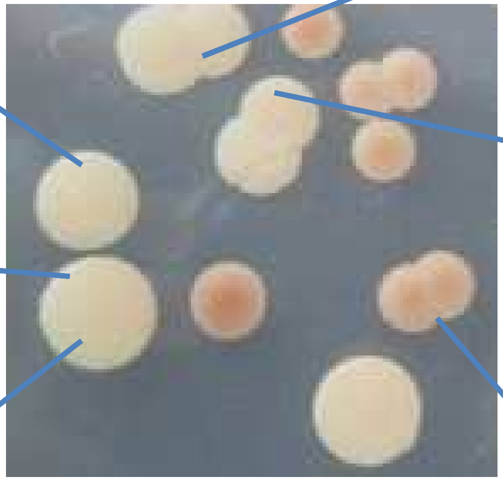
Merlot at 14°C from T=0 to 96h, then increase to 24°C
Metschnikowia : 25g/hL at T=0, then *S. cerevisiae* in sequential inoculation at T=96H
Control *S. cerevisiae* : 25g/hL at T=0

T=0
inoculation
Control: *S. cerevisiae*
Gaïa: *Metschnikowia*

T=96H
Modality Metschnikowia :
S. cerevisiae inoculation

Raynal et al, 2014

Metschnikowia fructicola **Gaïa™**



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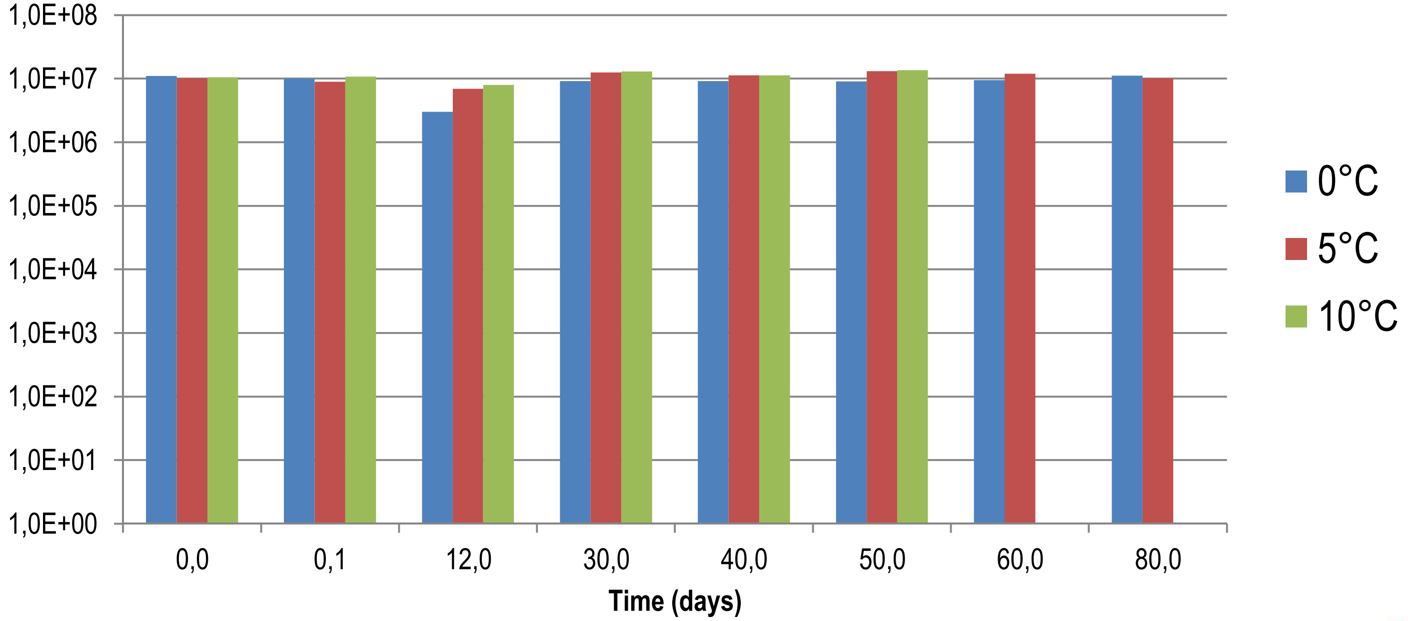
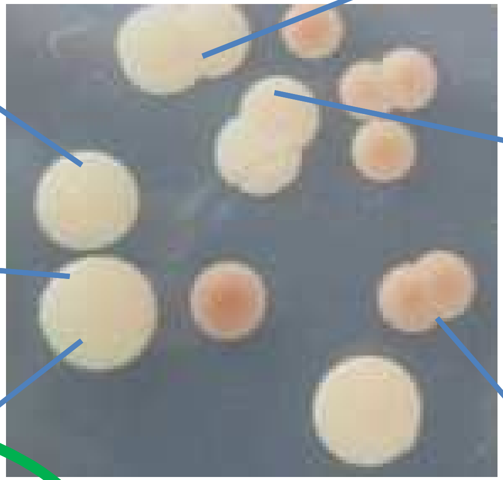
Implantation and survival of *M. fructicola* Gaïa™

- On must at low temperature and on long-term

Populations of *Metschnikowia* (cfu/mL) depending on the inoculation temperature – from 0 to 80 days (muscat stored at 0°C)



***Metschnikowia fructicola* Gaïa™**



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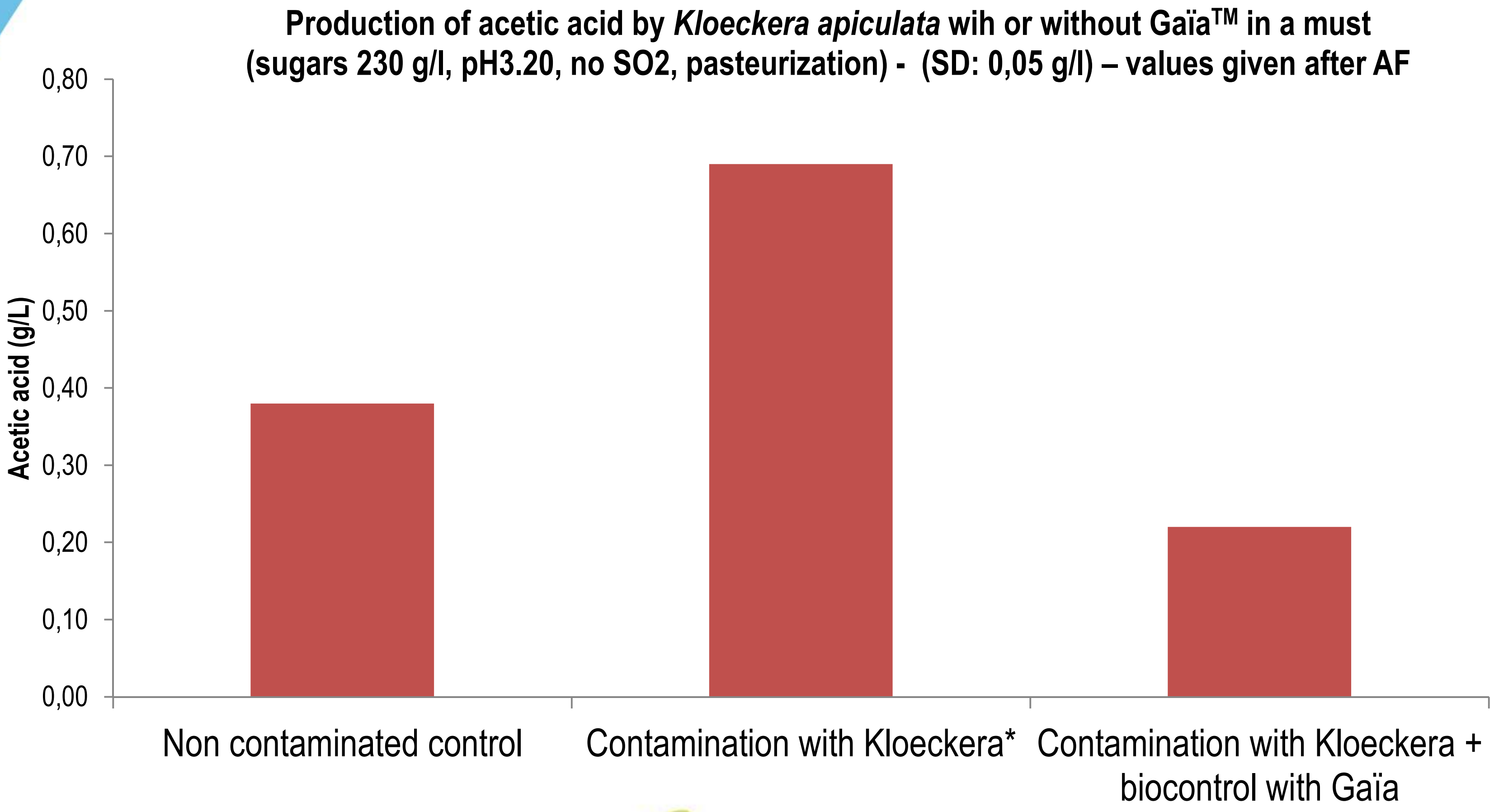
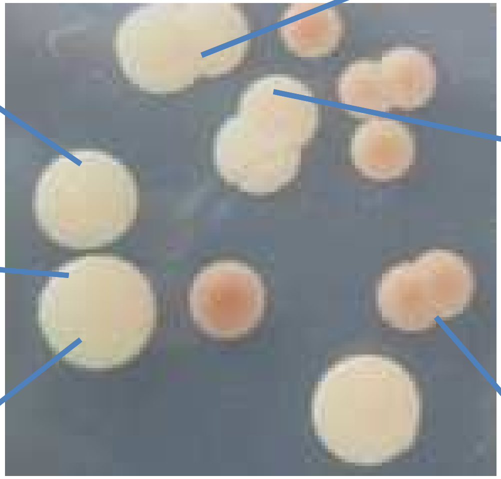
M. fructicola (Gaïa^{TM}): biocontrol against *Kloeckera* and volatile acidity



Gerbaux et al, 2015

Metschnikowia fructicola Gaïa™



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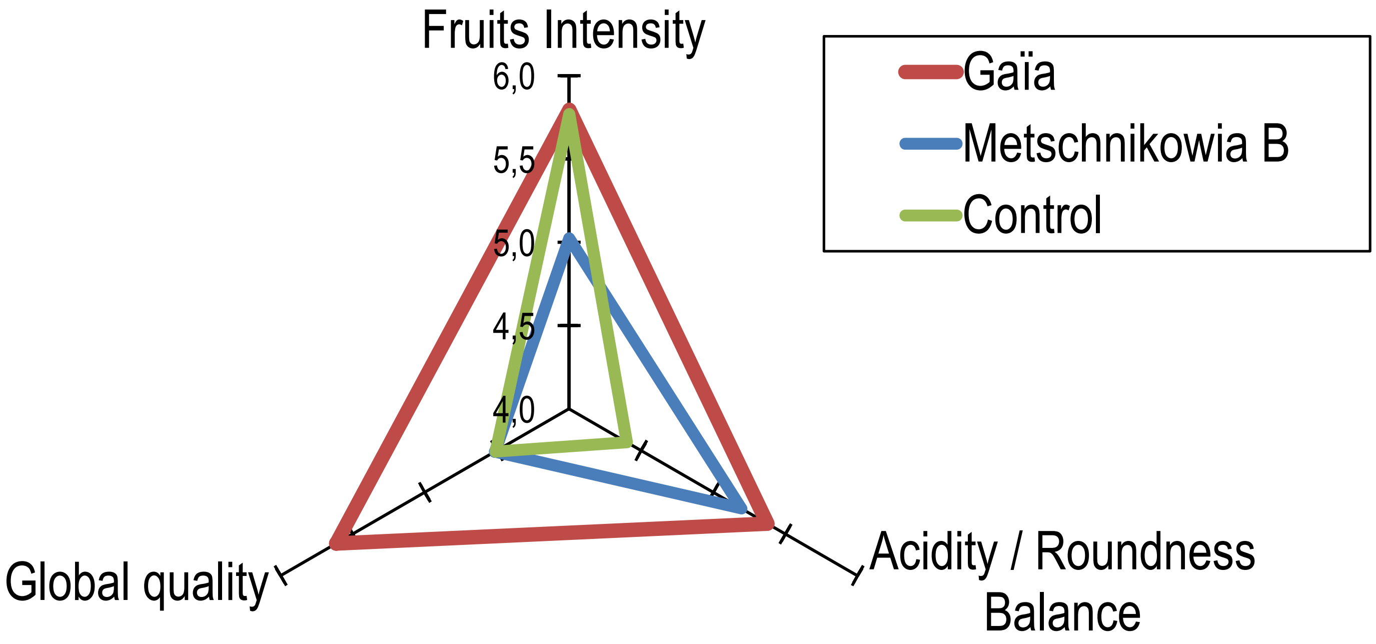
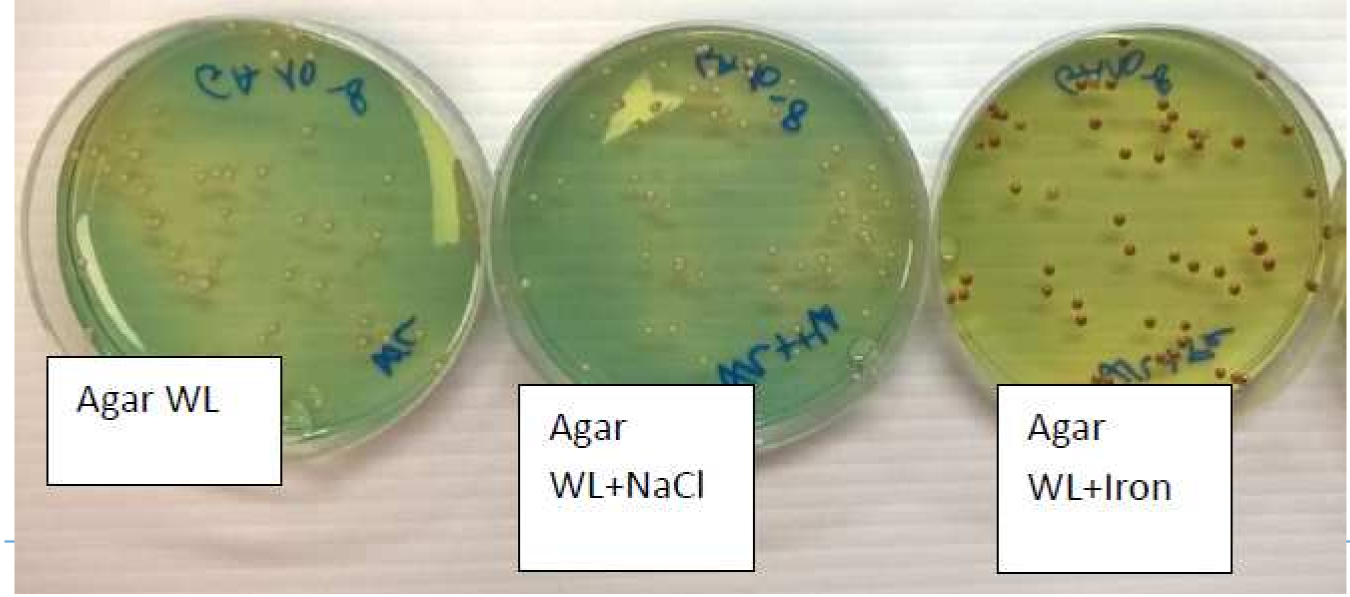
M. fructicola (Gaïa^{TM}): biocontrol and sensory contribution in cold soak

Sensory evaluation at the end of ageing of a red wine (pinot noir) in tanks of 2,5 hL with cold soak, with o without *Metschnikowia* - *Average values on 2 years.*



Gaïa: a tool of biocontrol against *Botrytis cinerea*

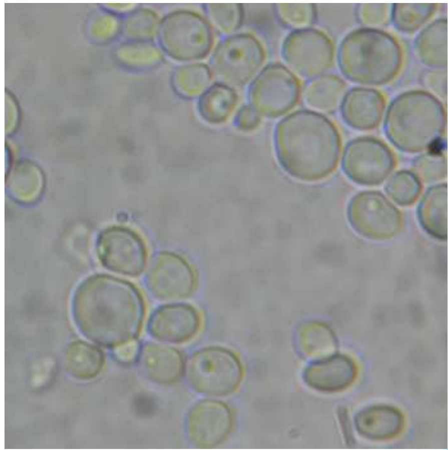
- *B. cinerea*: contaminating agent on desiccated grapes
- Gaïa fights actively against it :
  - Production of pulcherriminic acid



S. cerevisiae

Metschnikowia fructicola Gaïa™
Preservation of desiccated grapes (raisining)
Competitive asset

- Goal: to limit the post-harvest growth of *Botrytis cinerea*, for desiccated grapes.



Perdomini IOC
Vediamo diversamente.

DISAFA
Università degli studi di Torino

Università degli Studi di Verona

Unione Italiana Vini
since 1895

microbion
OPEN INNOVATION IN AGRI-FOOD MICROBIOLOGY

IOC
Révélons votre différence

Metschnikowia fructicola Gaïa™
Preservation of desiccated grapes (raisining)
Competitive asset

Gaïa 50 g/ ql: 41 days of desiccation

IOC
BE
LOw SO_2
SOLUTIONS

Management of SO_2 and SO_2-binding compounds

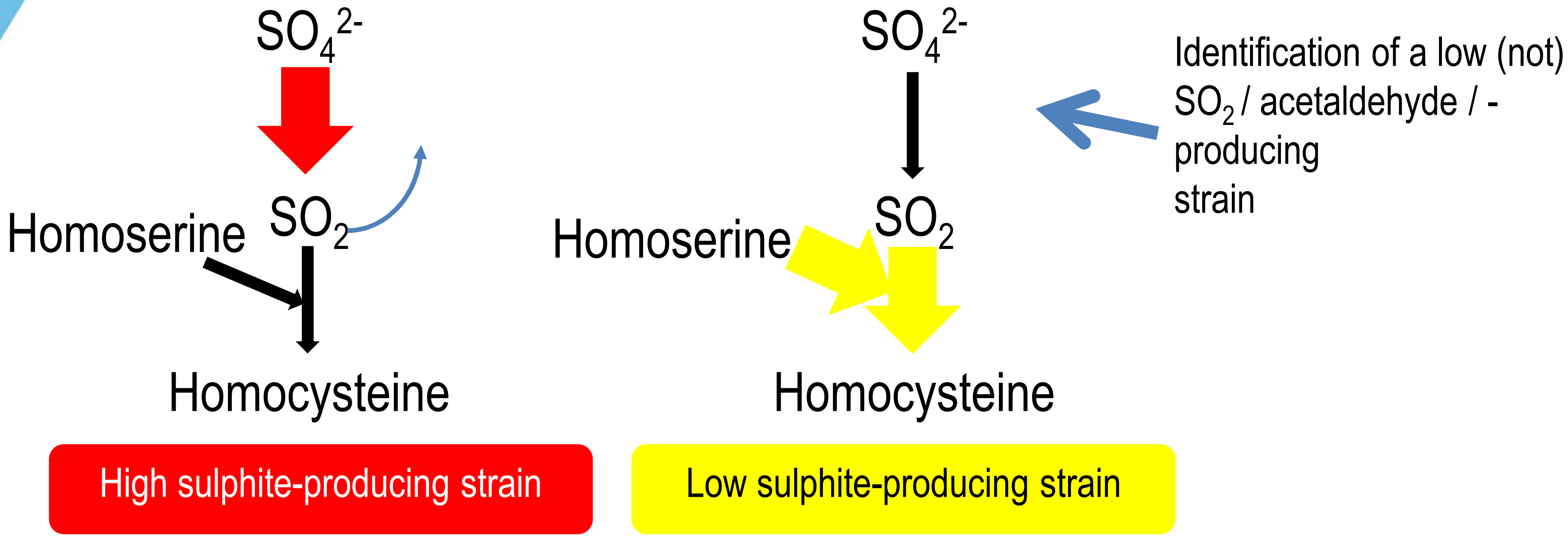
IOC BE YEAST: FERMENTATIVE BIOCONTROL

IOC BE yeast : interest

- Guaranteeing a tool for controlling SO_2 levels in wines:
  - By zero production of SO_2, and independently of the conditions
  - By a very low production of acetaldehyde, which combines SO_2

- Consequences:
  - “clean” wines

How does yeast work with sulfates and sulphites ?



SO_4^{2-}

Homoserine SO_2

Homocysteine

High sulphite-producing strain

SO_4^{2-}

Homoserine SO_2

Homocysteine

Low sulphite-producing strain

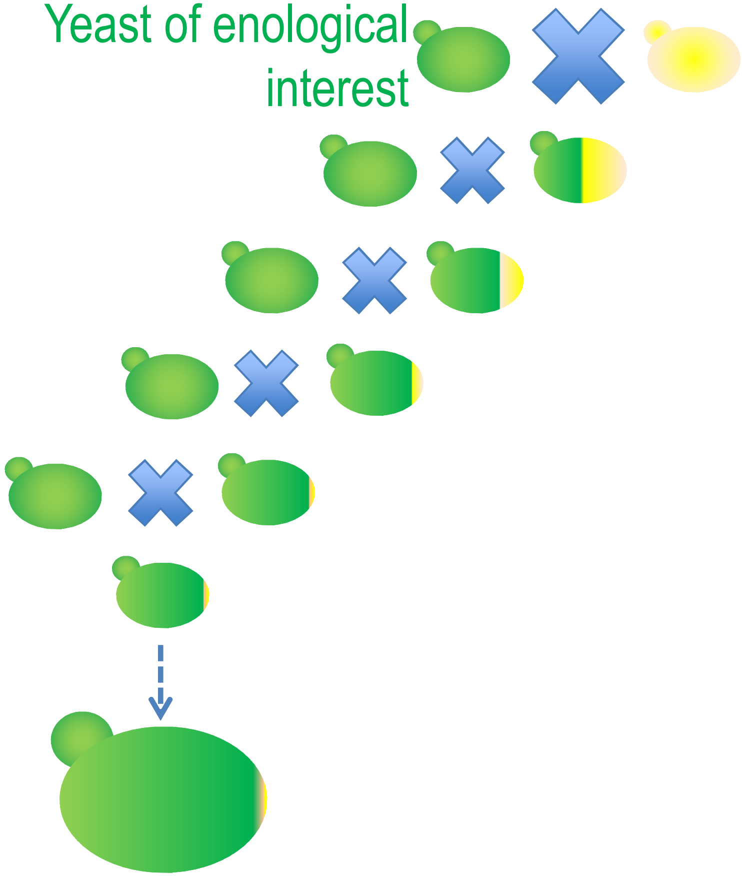
Identification of a low (not) SO_2 / acetaldehyde / - producing strain

Increased metabolism if:

- High NH_4+
- Low temperature
- Presence of sulfates
- Addition of sulphites in must

Birth of a tool to decrease sulphites in wine

Breeding Enhancement



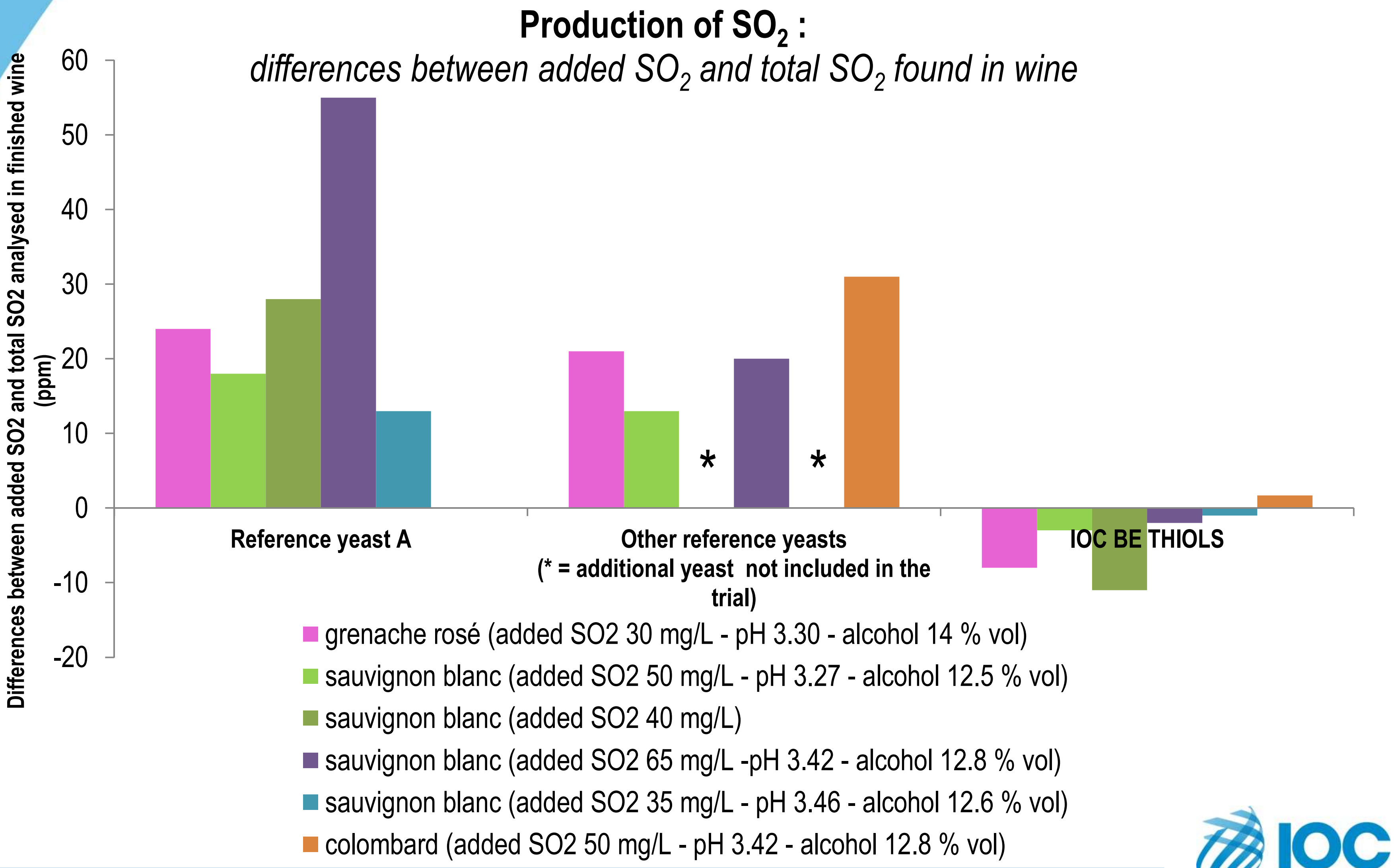
Yeast of enological interest

« Low SO_2 » mother-yeast

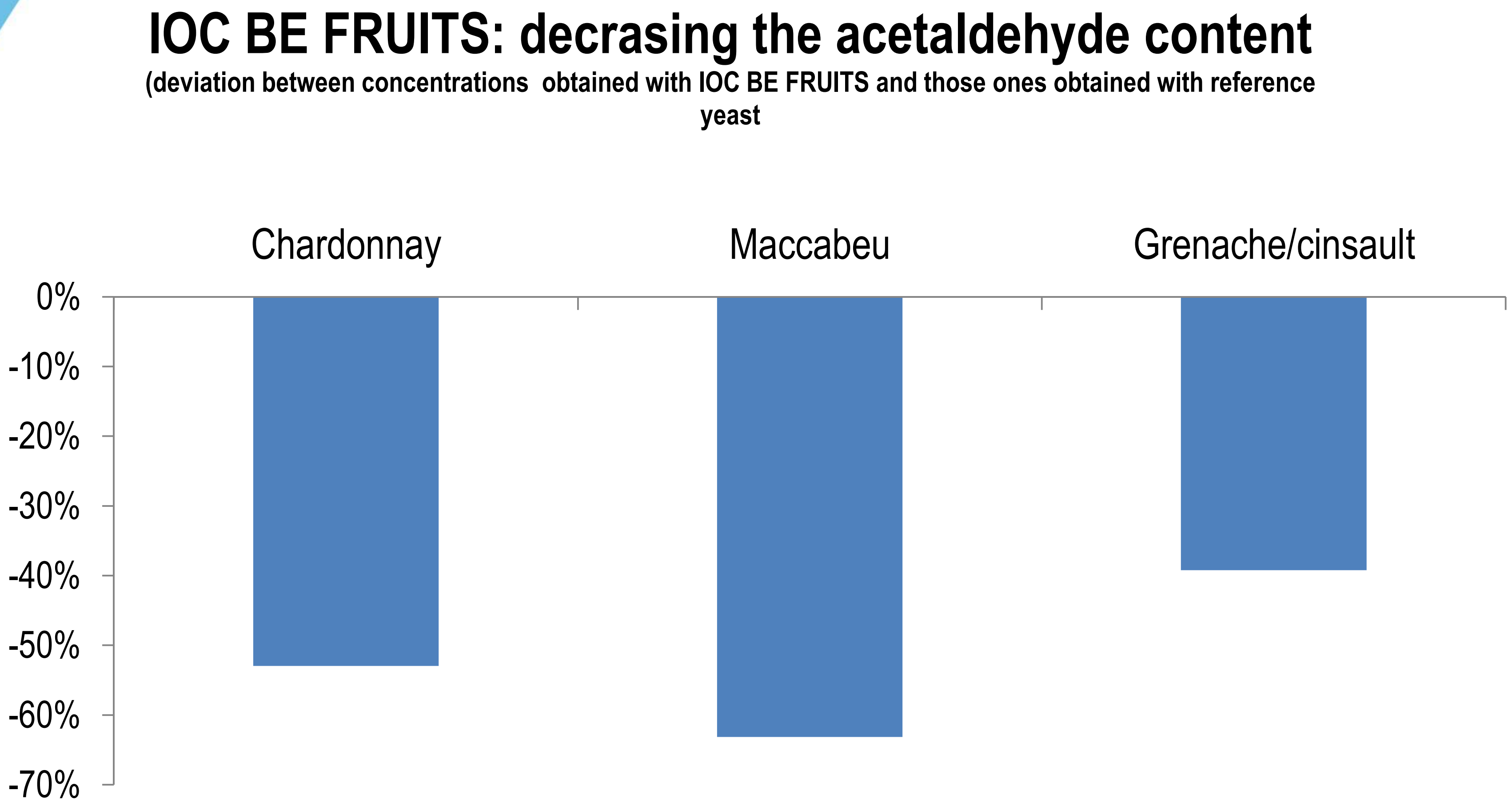
→ Successive back-crossings

→ Results (after a lot of oenological validations): a new yeast, containing a big part of the heritage from IOC yeast of enological interest, but with the guarantee of no production of SO_2 whatever the external conditions are.

Zero production of SO_2 whatever the conditions are

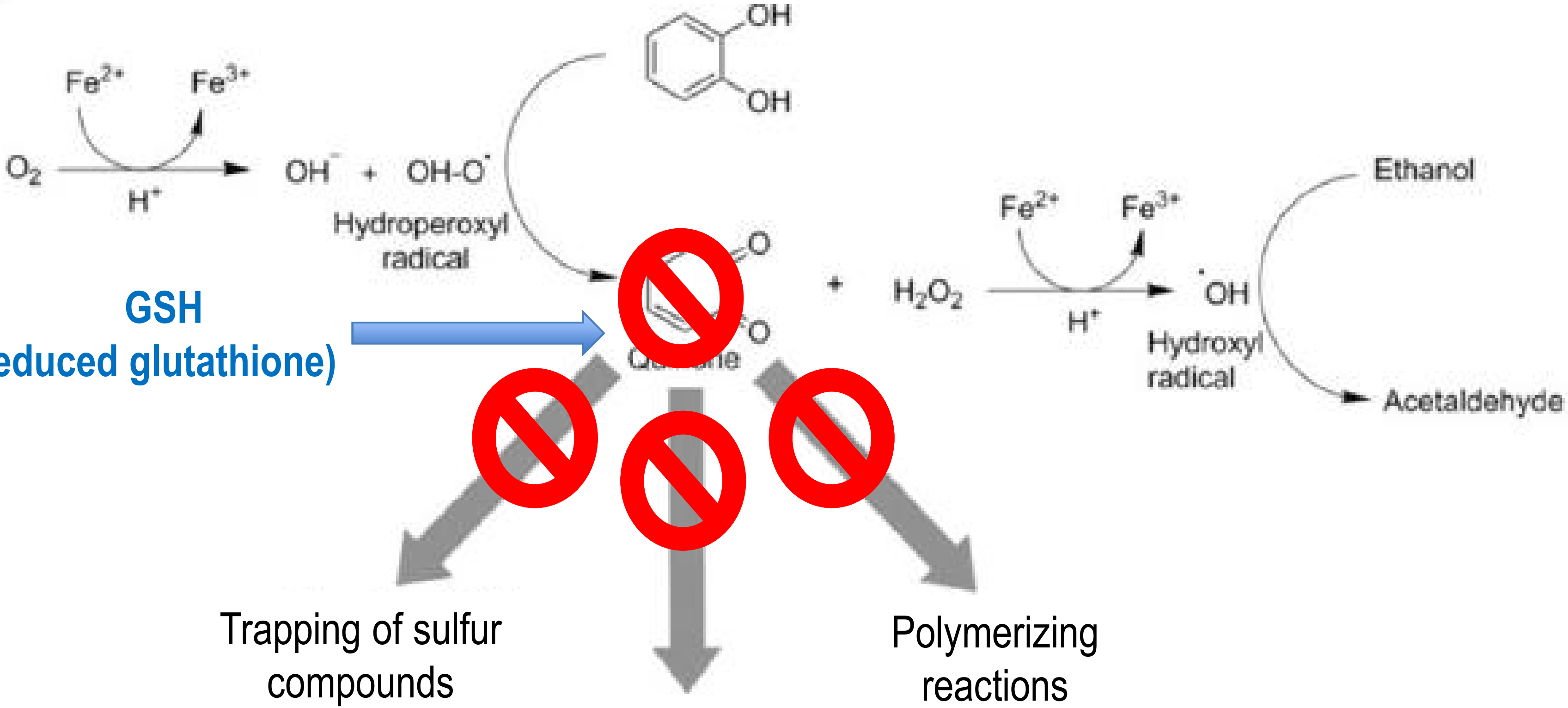


Low production of acetaldehyde – less bound SO2



LEES AND PRESERVATION OF WINE QUALITIES

Mechanisms of oxidation



GSH
(reduced glutathione)

Trapping of sulfur compounds (including fruity thiols)

Formation of aldehydes through Strekker degradation

Polymerizing reactions (browning)

Anticipating the protection against oxidation: the impact of inactivated yeast rich in glutathione

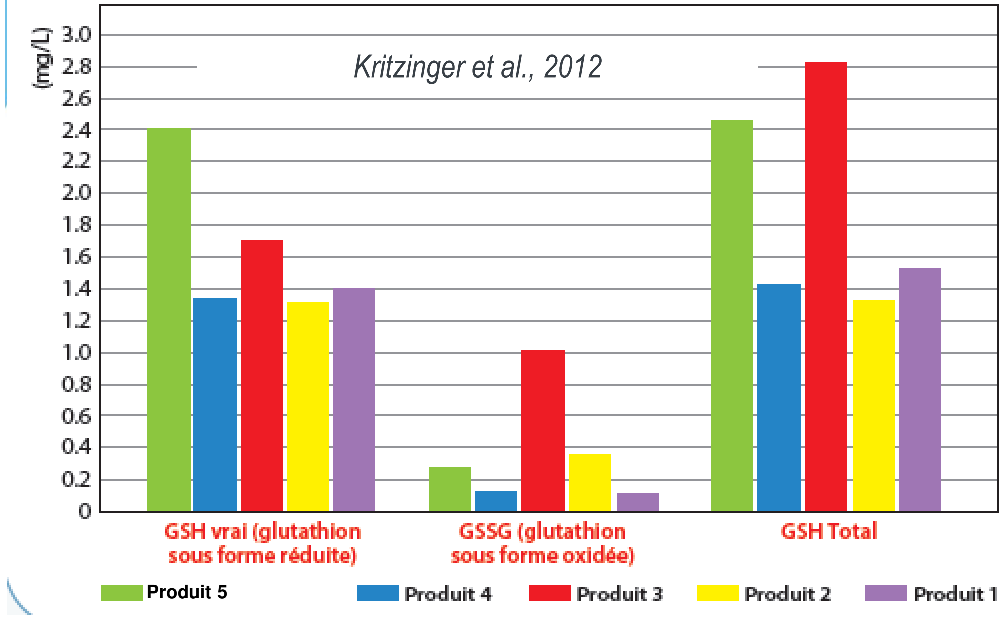
- Principle : Optimizing richness in antioxidants in musts and specially in wines

- Formulation : Specific inactivated yeast, naturally riche in reduced glutathione

- Goals :
  - Increasing biodisponibility of reduced glutathione in wines (and must) in order to induce the resistance of aromas to oxidation.

Pay attention to the different chemical species of glutathione!

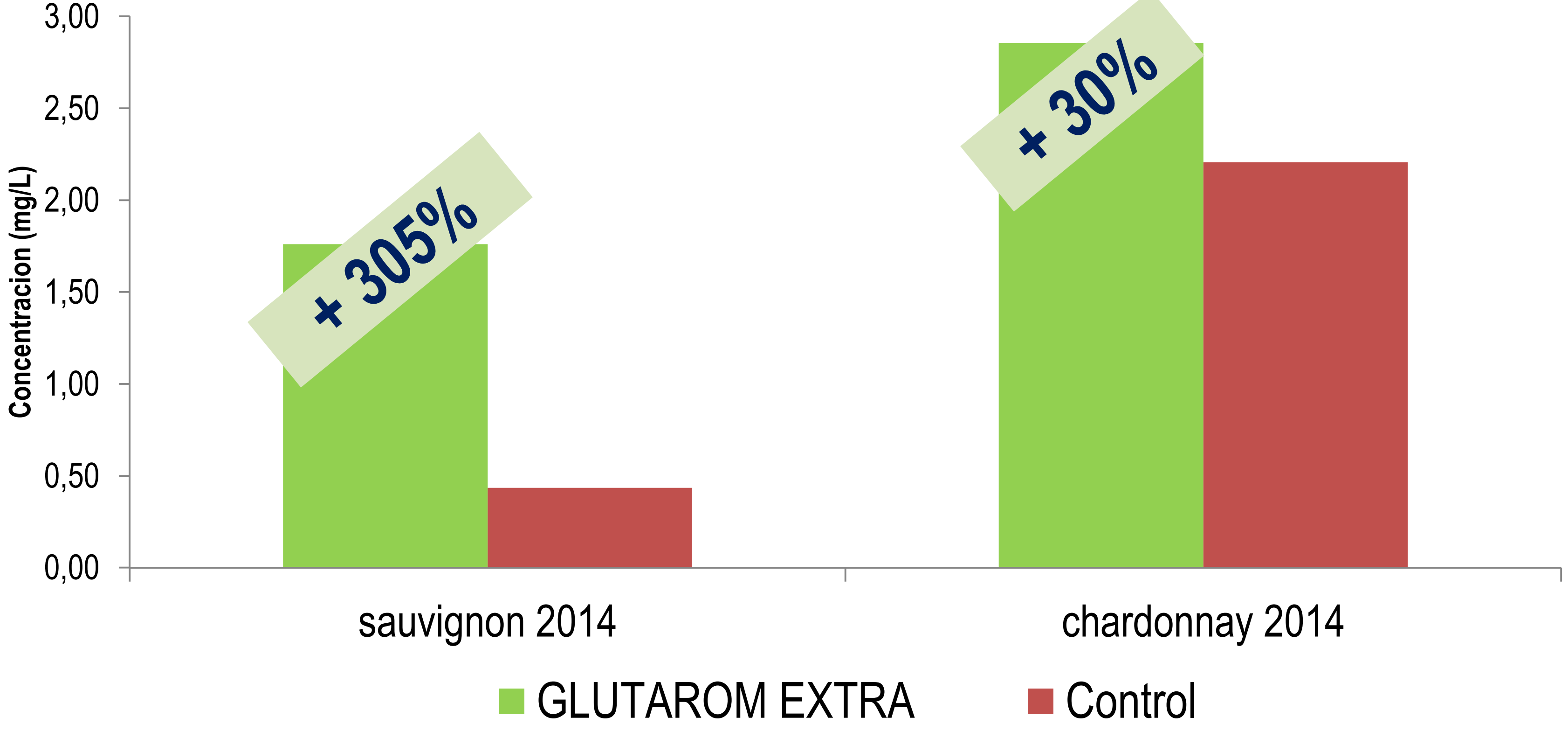
- Optimization of production process in order to increase the synthesis of GSH by yeast before inactivation
- Optimization of the content in reduced (or true) glutathione compared to total glutathione (GSH+GSSG (oxidized glutathione)).



Amounts in reduced, oxidized and total glutathione of different inactivated yeast naturally rich in glutathione.

Despite an apparently higher concentration in total glutathione, product N°3 is however less rich in reduced glutathione, the only one efficient to protect wine against oxidation.

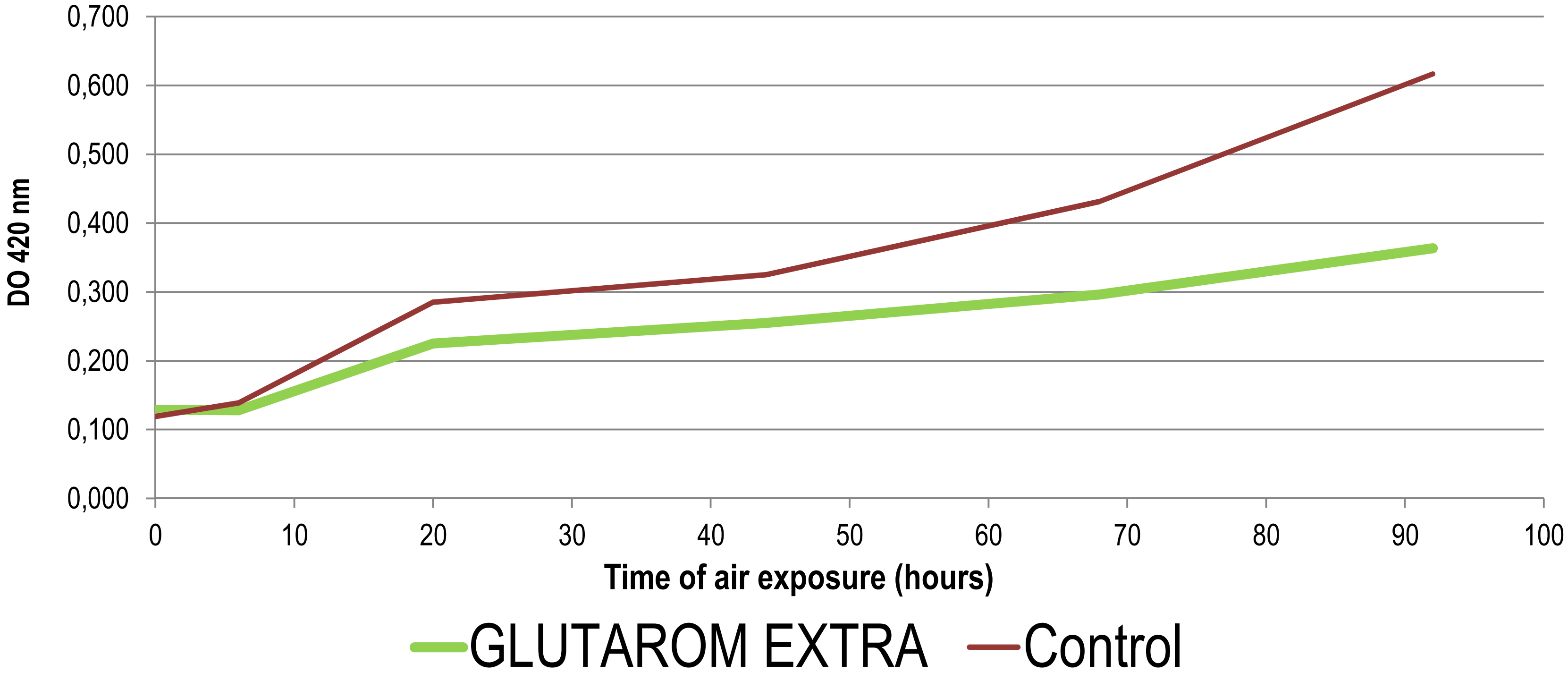
Alternatives to lees to protect wines against oxidation



A better resistance to air exposure

Low SO2 conditions: evolution of yellow colour during air exposure - chardonnay 2014 – analysis after AF after SO2 addition

SO2 additions: on must: 0 g/hL – wine post AF + before bottling : 0,4 g/hL



Anticipating the richness in reduced GSH

- In low SO2 wines, GLUTAROM EXTRA permits **amounts in GSH similar or higher** than the ones obtained with a full dosage of SO_2 addition (added at the settling of the must then post AF).
- These results are obtained with an addition at the **beginning of AF**.

Glutarom

Thanks for your attention !

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SULPHITE CONTENT

LOw SO$_2$
SOLUTIONS

Bioprotection,
Vinification, Storage

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