WORKSHOP
ORWINE: Scientific Basis for
European Rules on Organic Wine

Winemaking techniques to reduce sulfites and other additives

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Overview

- Tasks and aims of Work package 3 (WP3)
- Research results WP3
- SO_2-levels in organic wines from competitions
- Conclusions

Aim of Research Trials within WP3

- lowering of sulphite and other additives

through the whole wine production

by additional use of alternative and/or synergistic practices

4 GOALS of SO_2-addition in winemaking

- antimicrobial effect
- antioxidant effect
- inactivation of enzymes
- reaction with polar organic carbonyl compounds (SO_2-binding compounds ➡ increase of bound SO_2)

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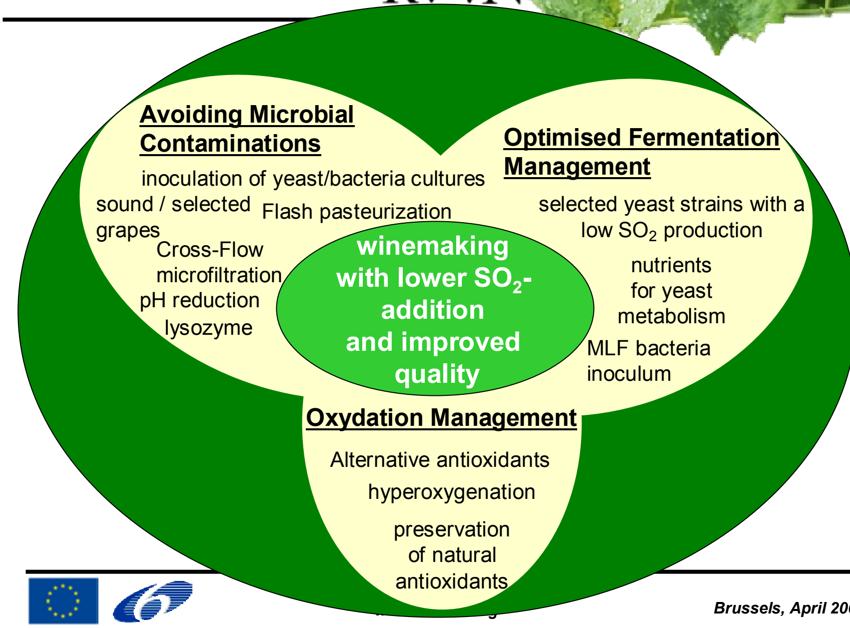
Influence on SO_2-Levels

- vintage
- grape variety
- sanitary conditions of grapes
- composition of spontaneous yeast population
- selected yeast strain
- nutritional conditions
- ...

Effects of Climate Change

- higher microbial infection, non traditional diseases
- higher sugar levels
- low acidity and higher pH-values
- nutrient deficiencies
- earlier harvest period

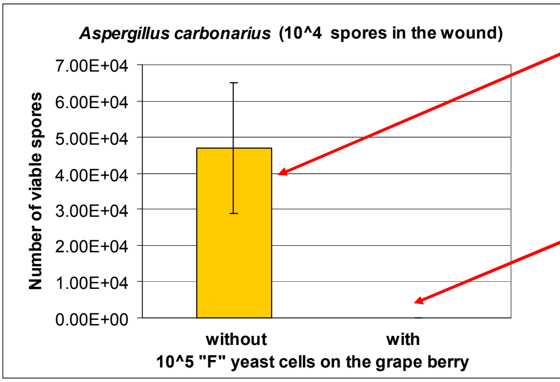
increasing need of SO_2-addition in certain vintages and specific wine growing areas!



Avoiding of Microbial Contamination

- **wine yeast spraying on grapes** to avoid microbial contamination
- inoculation of **appropriate yeast and lactic acid bacteria starter cultures** to overwhelm indigenous microbial population with high SO_2 production and/or negative for wine quality
- use of **Flash-pasteurization (FP)** and **Cross-Flow microfiltration (CF-MF)** to stop contamination
- wine **pH reduction** by addition of tartaric acid or techniques like polar membrane electrodialysis to slow down contaminant growth
- use of **lysozyme** to avoid lactic acid bacteria contamination

Effect of simultaneous yeast inoculation on infected damaged berries



Aspergillus carbonarius

Strong effect on *A. carbonarius*

Source: INRA

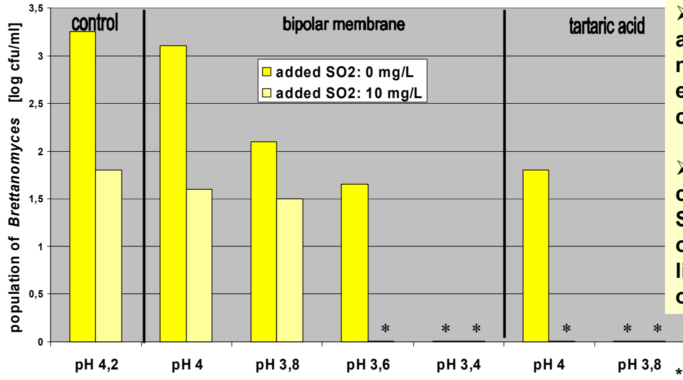
Yeast Starter Culture & Lysozyme White Wines

PINOT GRIS	Volatile acidity (g/L)	Malic acid (g/L)	Lactic acid (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Acetaldehyde (mg/L)
Classic inoculation SO_2	0,19	2,56	0,13	8	14	13
Active yeast starter NO SO_2	0,18	2,59	0,25	1	2	5
Active yeast starter + lysozyme NO SO_2	0,18	2,75	0,28	4	5	6

End of alcoholic fermentation:

- ***wines without SO_2 additions have same VA and analytical parameters***
- ***Less SO_2 binding compounds ⇒ lower later SO_2 additions***

Decrease of *Brettanomyces* Population



Source: ITV

- addition of tartaric acid and bipolar membrane electrodialysis (ME) can lower wine pH
- lower pH and consequent more free SO_2 can inhibit growth of contaminant yeasts like *Brettanomyces sp.* or lactic acid bacteria

- ***tartaric acid addition (allowed) is equally effective***
- ***ME not yet authorized by EU regulation***

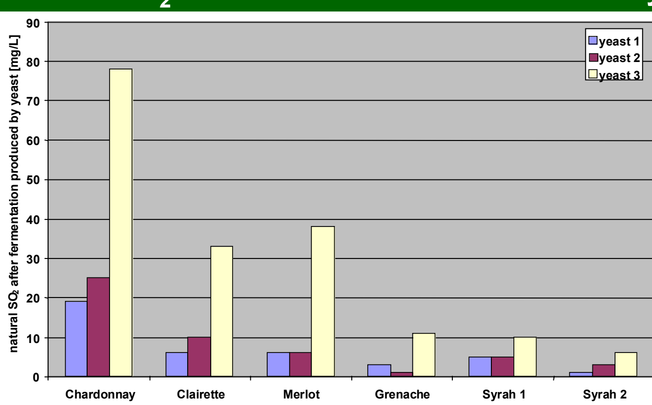
Avoiding contamination — Main conclusions

- Use of activated **starter cultures** can avoid growth of spoilage microorganisms in must
- **co-inoculum** of lactic acid bacteria during alcoholic fermentation can be an useful tool to obtain malate degradation while avoiding microbial spoilage
- **lysozyme** can be useful to limit lactic bacteria growth (in starter culture and/or wine)
- **CFM and Flash Pasteurization** can be as effective as SO_2 addition in stopping yeast and bacteria contamination
- **pH reduction** through bipolar membrane is effective but presently not advisable in organic wine production; alternative addition of **tartaric acid**
- New research: **yeast spraying** on grapes before harvest

Optimised Fermentation Management

- selecting yeast starter cultures with a low SO_2 production
- suitable nutrient supplementation for balanced yeast metabolism
- inoculation of lactic acid bacteria starter cultures

Natural SO_2 after Fermentation Produced by Yeasts



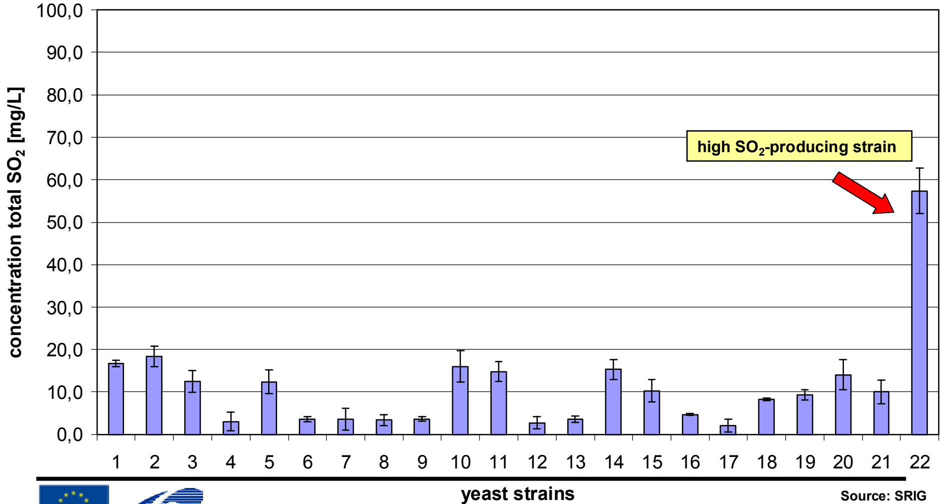
Source: ITV

Trial type: musts from 5 different grape varieties on the same location

fermented with 3 different commercial strains of wine yeasts

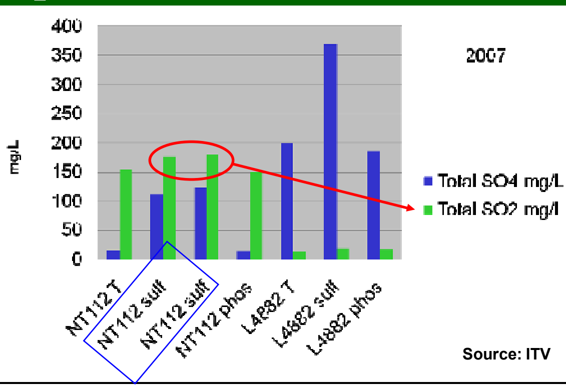
Important differences in SO_2 production depending on the grape variety. Some yeast strains consistently produce more SO_2 – in all situations !

Low SO_2 producing yeasts recommended by yeast companies



Source: SRIG

SO_2-Production & ammonium salts



Source: ITV

Chardonnay fermented with different fermentation activators

Two yeasts with high (NT112) and low (L4882) producing "natural SO_2"

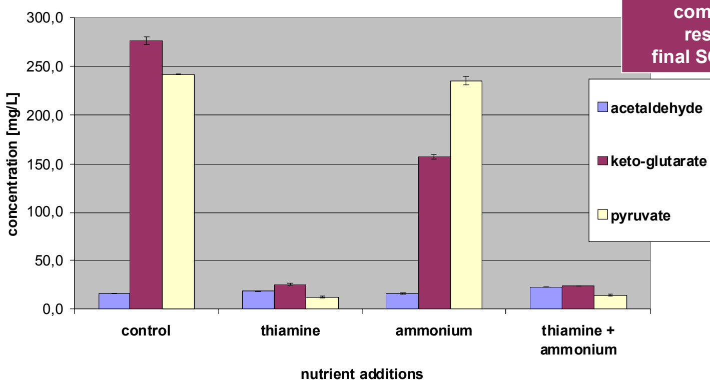
Winemaking without SO_2 before the end of alcoholic fermentation

NT112 produces more SO_2 than L4882.
SO_4 coming from ammonium sulfate can be used to produce more SO_2.
Strain L4882 seems to be not able to use SO_4.

Formation of SO_2-binding compounds

Formation of SO_2 binding compounds during fermentation and the influence of nutrients (n=3)

The formation of SO_2 binding compounds is mainly responsible for the final SO_2 need of the wine.



Thiamine is indispensible if the SO_2 concentration of the wine should be lowered.

Optimised Fermentation Management

Main conclusions

- Alcoholic fermentation must be dominated by **yeasts strains with low SO_2 production** → use of selected strain
- preference for **phosphate ammonium** salts vs sulphate
- **thiamine** and micronutrients important to decrease production of SO_2-binding compounds

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Oxydation Management

- white grape must hyperoxygenation to reduce wine sensitivity to oxidation
- addition of alternative antioxidants like ascorbic acid and tannins
- preservation of natural antioxidants from grapes and yeasts (e.g. glutathione)

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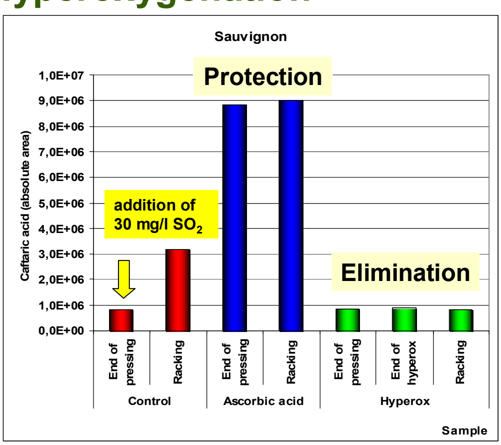
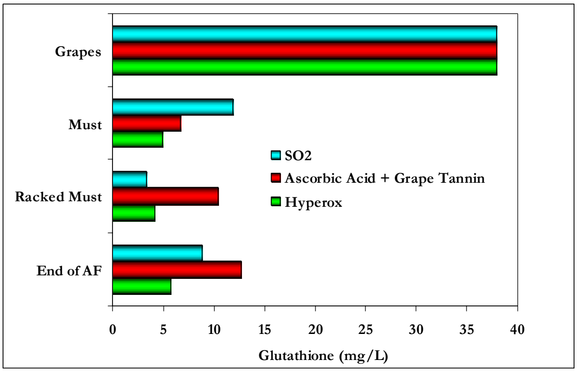
Grape Tannins & Ascorbic Acid vs. Hyperoxygenation



Caftaric acid is one of the first compounds to be oxidized in white grape must. Less caftaric acid indicates that more oxidation occurred.

- *tannins + ascorbic acid avoid must oxidation*
- *effective on Sauvignon blanc, much less on Pinot gris*

Preservation of Natural Antioxidants (e. g. Glutathione)



Glutathione is a sulphur-containing antioxidant naturally present in grapes and wine yeasts.

Addition of ascorbic acid (Vitamin C) and grape tannins to the grape juice (instead of SO_2) preserves greater quantities of glutathione in the wine

Avoiding Oxydation

Main conclusions

- **hyperoxygenation:** suitable practice for stabilizing white wines; carefully used in aromatic varieties sensitive to oxygen
- **reductive winemaking:** preferred practice for wines with evident varietal traits
- **ascorbic acid** and **tannins** in juice can partially replace SO_2, by limiting oxidation and by preserving natural antioxidants

Survey on SO_2 levels in Organic Wines from Competitions

SO_2 levels detected in organic wines from competitions

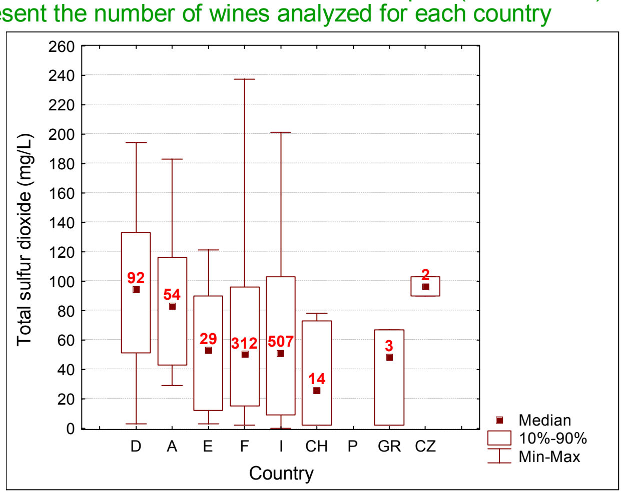
- Survey on sulfur dioxide levels in 1.014 wines from organic viticulture
- European "Organic Wine" Competitions: Biodivino (Italy, 2006-2007), Millesime Bio (France, 2007-2008), Biofach (Germany, 2007-2008), and Ecovin Competition (Germany, 2006)
- Sulfur dioxide was determined by the official EU method, as reported in the EU Regulation 2676/90

Sulfur dioxide limits reported in the EU Regulation 1493/99

Residual Sugars	< 5 g/L		> 5 g/L	
Wine Type	White	Red	White	Red
Sulfur Dioxide Limit (mg/L)	210	160	260	210

Exceptions (300, 350 or 400 mg/L) for special wine categories, or particular climatic conditions

Sulfur dioxide levels detected in the collected samples (1.014 wines); red labels represent the number of wines analyzed for each country



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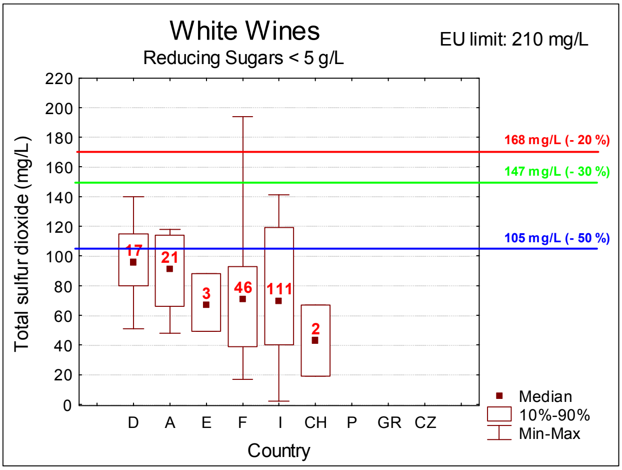
Sulfur dioxide levels in the collected white dry wine samples (residual sugars lower than 5 g/L); red labels represent the number of wines analyzed for each country



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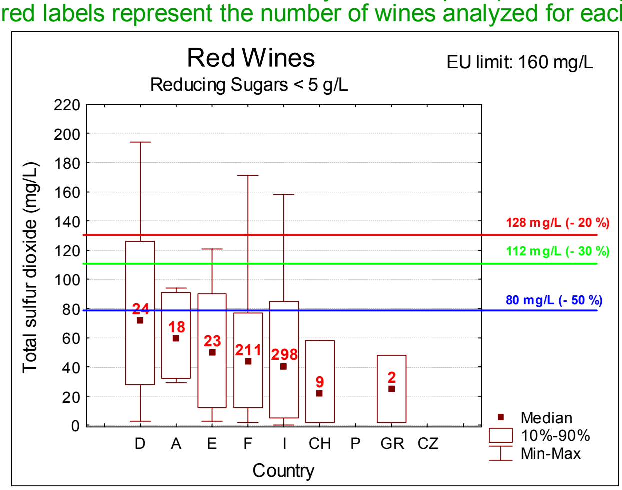
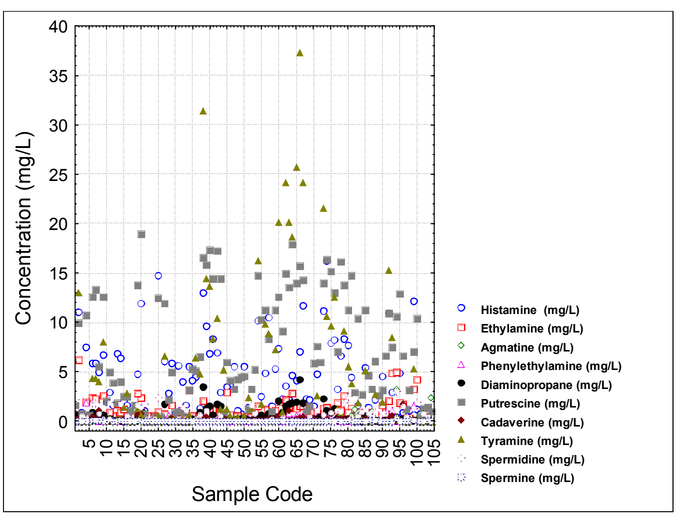
Sulfur dioxide levels in the collected red dry wine samples (residual sugars lower than 5 g/L); red labels represent the number of wines analyzed for each country



ORWINE survey and wine pollutants

- Ochratoxin A (OTA)
- 204 samples from competitions analyzed
- Only 10 of them showed OTA level higher than 2 μg/L (current EU limit - EC Reg. 123/2005)
- Not generalized risk for OTA pollution
- These wines were particularly related to specific regions (e.g. south Italy)

Levels of biogenic amines detected in 105 wine samples collected during the Competitions mentioned before. Results of ORWINE survey



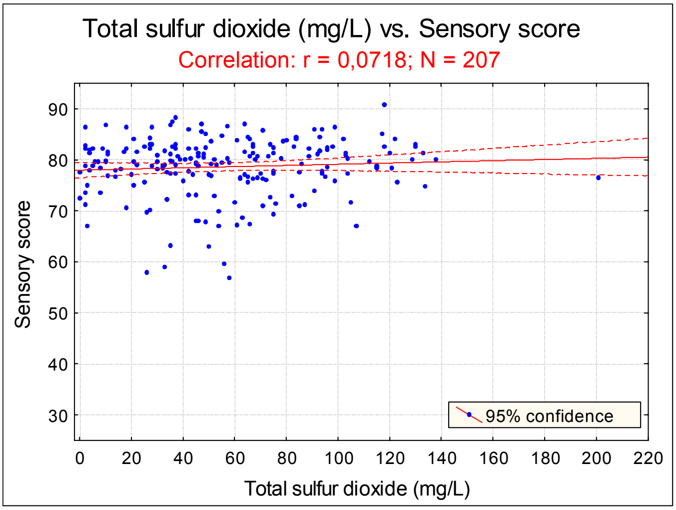
- Biogenic amines and wine composition
  - Poor correlations with free and total SO_2
  - Higher levels in wines with incomplete malolactic fermentation
  - Higher levels in wines with high pH and volatile acidity
  - Problems in the management of alcoholic and malolactic fermentations

Effect of co-inoculation on BA control

Sample	Tyramine (mg/L)	Putrescine (mg/L)	Total (Free) SO_2 (mg/L)
Classic inoculation SO_2	**0,8**	**1,9**	**16 (4)**
Classic inoculation NO SO_2	**1,3**	**5,2**	**1 (*n.d.*)**
Co-inoculation NO SO_2	**0,8**	**2,8**	**1 (*n.d.*)**

Histamine: not detectable
Merlot 2006: alcoholic strength 12,00 % v/v; samples collected during on lees storage (23/01/2007)

Sulfites and wine sensory characters (WP3)



Sulfites reduction protocols and wine sensory characters (WP3)

- Wine sensory characters were assessed for each protocol tested for SO_2 reduction in WP3; some examples
  - Hyperoxygenation vs. SO_2: no significant differences except for specific varieties very sensitive to oxygen (e.g. Sauvignon)
  - Ascorbic acid + tannins vs. SO_2: no significant differences
  - Selected yeasts management (*pied de cuve*) vs. SO_2: wines obtained without using sulfites before AF, appeared slightly more oxidized but showed more intense flowery notes, more bodyness and higher score as regards overall judgment
  - Co-inoculation vs. SO_2: no significant differences on two years (2006 and 2007)

Implementation of WP 3 results on SO_2 reduction strategies during winemaking process

SO_2 reduction strategies

PRODUCTION STEP	PRACTICE	Lab or experimental scale results (WP3)	Tested in pilot wineries (WP4)	Remarks/Effect
Grape defence against moulds	Yeast spraying	**Positive**		Lower risk of OTA formation in wine, by inhibition of *A. carbonarius* infections on grapes
Grape processing	Reductive winemaking - addition of tannins and ascorbic acid	**Positive**	YES	Reduction of oxygen activity in juice and consequently good alternative to SO_2 addition for musts protection
Juice processing	Hyperoxygenation	**Positive**	YES	Stabilization of grape juice by oxidation of unstable phenolic compounds; indicated for some varieties

SO_2 reduction strategies

PRODUCTION STEP	PRACTICE	Lab or experimental scale results (WP3)	Tested in pilot wineries (WP4)	Remarks/Effect
Alcoholic fermentation	Use of selected yeasts	**Positive**	YES	Reduction of the dominance of wild microorganisms or high SO_2 producing strains
	Activated yeasts starter cultures	**Positive**	YES	Prevention of microbial contamination by giving competitive advantage to selected yeasts
	Thiamine supplementation	**Positive**	YES	Reduction of the formation of SO_2 binding compounds - higher free SO_2 available
	Ammonium phosphate addition	**Positive**	YES	Good yeast activity and reduction of the risks of microbial contamination
	Ammonium sulphate addition	**Negative**		Can be metabolized by yeasts to produce SO_2, better to use ammonium phosphate
	Use of lysozyme	**Positive**	YES	Reduction of lactic bacteria contamination; useful in high pH juices and wines, where SO_2 is less effective

SO_2 reduction strategies

PRODUCTION STEP	PRACTICE	Lab or experimental scale results (WP3)	Tested in pilot wineries (WP4)	Remarks/Effect
Malolactic fermentation	Use of selected bacteria	**Positive**	YES	Correct management of MLF
	Co-inoculation yeasts – lactic bacteria	**Positive**	YES	Prevention of microbial contamination, reduced biogenic amines formation even with low SO_2
Wine storage & preparation	On lees storage	**Positive**	YES	Limitation of oxidations; risk of BA pollution if MLF is incomplete or occurs spontaneously
	Bipolar membrane treatment	**Positive**		Effective in pH reduction, and higher (more favourable) free/total SO_2 ratio, but not allowed and expensive; HTH addition is equally effective
	Cross-flow microfiltration	**Positive**		Physical elimination of microorganisms
	Flash-Pasteurization	**Positive**		Physical elimination of microorganisms
	Glutathione (GSH) supplementation/ Preservation	**Positive**		Antioxidant effect, not allowed (useful: strategies for the preservation of natural GSH contents)

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Ongoing Research Recommended

•Additional experiences are needed to evaluate long ageing and stability of wines prepared with specific oenological tools

• An eventual restriction of SO_2 limits for organic wine should consider the normal operating conditions in the different winemaking areas, but also:

- climatic conditions
- seasonal events
- “winemaking habits”…

Summary and Conclusions

- Reduction of SO_2 (before, but also after alcoholic fermentation) is technically possible with certain techniques and alternative additives which are in tune to organic principles and are admitted by both CMO and organic regulations, and accepted by the winemakers
- The effect of certain oenological tools on SO_2 is influenced by grape variety, grape must composition and wine type
- Potential for implementing SO_2 reducing strategies is higher in pre-fermentative steps than during wine storage and ageing

Summary and Conclusions

- Strategies to reduce SO_2 binding compounds offer a more favorable ratio between free and total sulfur dioxide, and lead to a better efficiency of each SO_2 addition

- new techniques were tested with positive results that are not yet allowed for conventional wines

  (yeast spraying, bipolar membranes …)

- An **optimized combination of oenological tools** is necessary (code of good practices; experiences WP 4) to maintain and to avoid a negative effect on wine quality