



THE CHALLENGE OF SULPHITE CONTENT REDUCTION IN ORGANIC WINES: possible strategies for red winemaking

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9th November 2020



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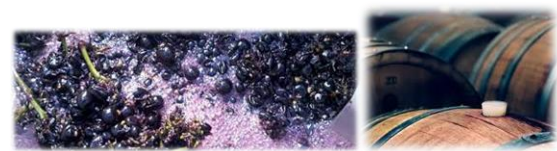




Summary



Sulfur dioxide - *properties*



SO₂ selects the must microflora and acts as an antimicrobial in wines

- apiculate yeasts
- acetic and lactic acid bacteria
- *Brettanomyces* spp.

- Solubilizing agent
- Fining agent
- Binding agent



Antiseptic

Antioxidant

SO₂

Other functions

Antioxidase

Chemical oxidation

Factors

Polyphenols
Oxygen
Metals
Radical species
Acetaldehyde

Enzymatic oxidation

Grape PPO:
Tyrosinase

Botrytis cinerea PPO:
Laccase



Sulfur dioxide– *To reduce or eliminate it?*



- Easy to use
- Easy to produce
- Cheap
- Effective



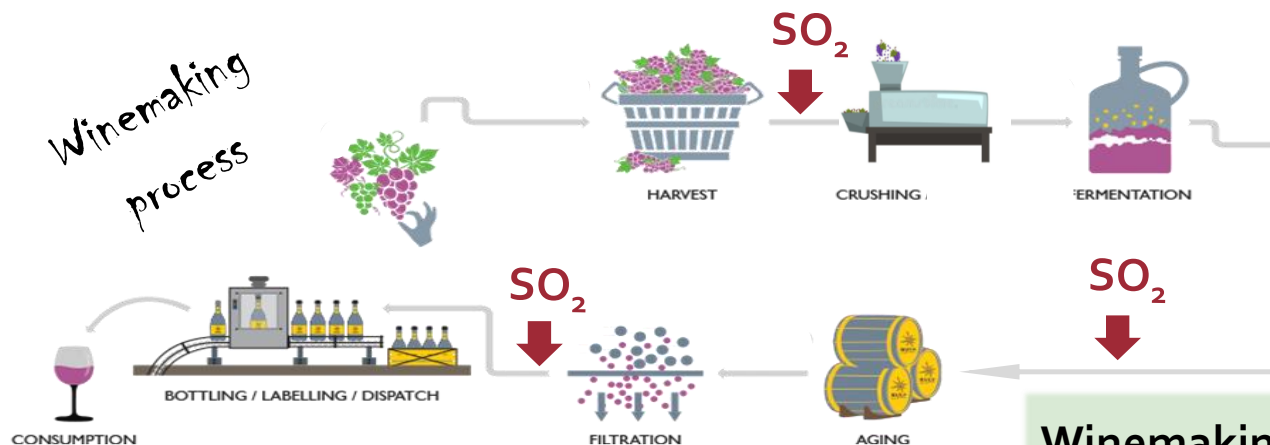
Health issues

- Allergy (1% of population)
- Intolerance
- WHO indication

Avoid SO₂ to improve the “naturalness” of wines



It is possible to eliminate sulfur dioxide or reduce its content?



EU SO₂ Limits red wines

Conventional (sugars ≤ 2 g/L) : **150 mg/L**

Organic (sugars ≤ 5 g/L) : **100 mg/L**

Winemaking practices

Alternatives additives

Physical treatments and processing aids

Sulfur dioxide - *how to increase effectiveness in winemaking*

Molecular SO₂ Factors:

Antiseptic

SO₂

- ↑ pH
- ↑ ethanol
- ↑ temperature

Bacteria : 0.5- 1 mg/L

Yeasts: 1 - 2 mg/L

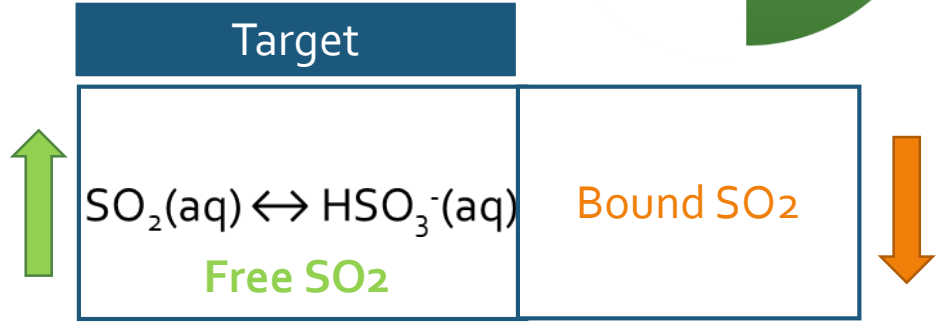
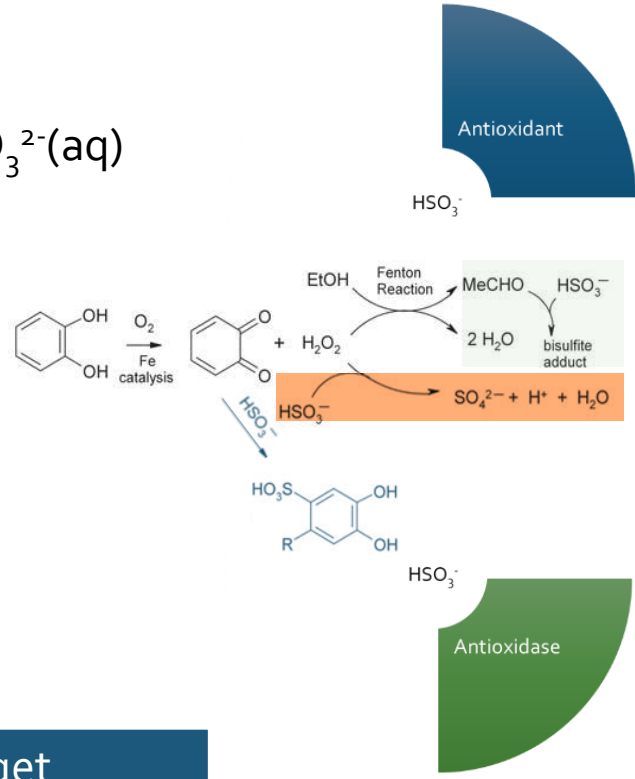
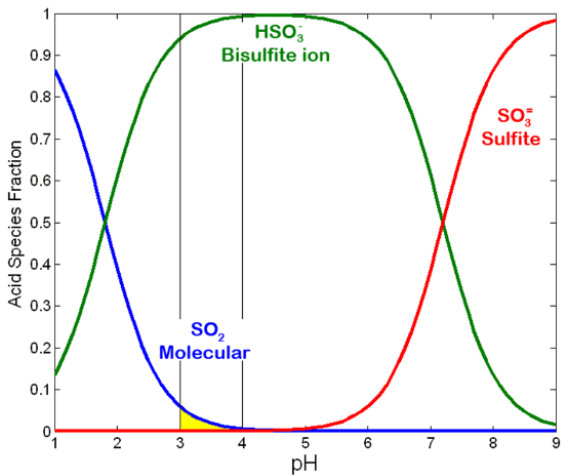
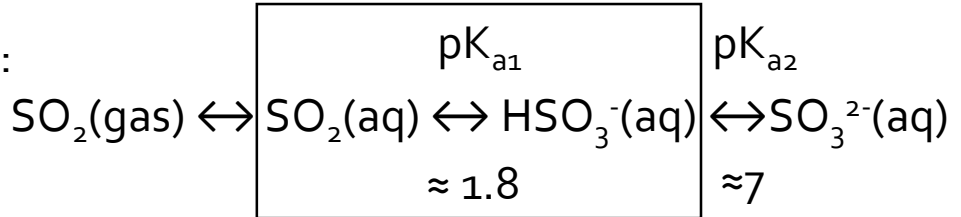
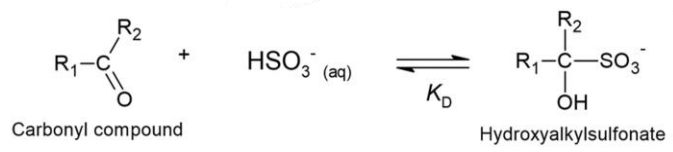
Other functions

HSO₃⁻

Binding

Acetaldehyde **irreversible**

Other acid and polyphenols **reversible**



SO₂ Alternatives



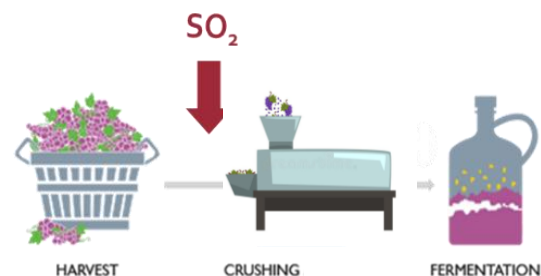
Table 2 Some alternatives of SO₂ used during wine production

A. Chemical Additives	B. Non-thermal Applications	C. Phenolic Compounds and Plant Extracts	D. Killer Toxins and Bacteriocins	E. Combine methods
DMDC (Costa and Loureiro, 2008)	High Hydrostatic Pressure (HHP) (Briones-Labarca <i>et al.</i> , 2017)	Eucalyptus and almond skin extracts (Garcia-Ruiz <i>et al.</i> , 2013b)	<i>Kluyveromyces phaffii</i> DBCG 6076 (Ciani <i>et al.</i> , 2001)	Glutathione and caffeic acid or gallic acid (Roussis and Sergianitis, 2008)
Lysozyme (Azzolini <i>et al.</i> , 2010)	Pulsed Electric Field (PEF) (Delsart <i>et al.</i> , 2015a,b)	Stilbens extracts (Raposo <i>et al.</i> , 2016b)	Pediocin PA-1 (Diez <i>et al.</i> , 2012)	Glutathione and/or elligatannis (Panero <i>et al.</i> , 2015)
Ascorbic acid (Sonni <i>et al.</i> , 2011)	Ultraviolet Irradiation (UV-C) (Fredericks and Krügel 2011)	Thyme essential oil (Freidman <i>et al.</i> , 2017)	Nisin (Rojo-bezares <i>et al.</i> , 2007)	Glutathione and ascorbic acid (Comuzzo <i>et al.</i> , 2015)
Ethanethiol (Dias <i>et al.</i> , 2013)	Ultrasound (HPU) (Jiranek <i>et al.</i> , 2008; Gracin <i>et al.</i> , 2016)	Grape and wood tannins (Sonni <i>et al.</i> , 2009; Alamo-Sanza <i>et al.</i> , 2019; Sánchez-Palomo <i>et al.</i> , 2017)	Killer toxins CpKT1 and CpKT2 (Mehlomakulu <i>et al.</i> , 2014)	Lysozyme and polyphenols (Chen <i>et al.</i> , 2015)
NaOCl (sodium hypochlorite) (Yoo <i>et al.</i> , 2011)	Dry Ice Application (Costantini <i>et al.</i> , 2015)	Hydroxytyrosol and oleuropein (Raposo <i>et al.</i> , 2016a,b)	Lacticin 3147 (Garcia-Ruiz <i>et al.</i> , 2013a)	Lysozyme and tannins (Sonni <i>et al.</i> , 2009)
Chitosan (Chinnici <i>et al.</i> , 2014; Elmaci <i>et al.</i> , 2015)		Glutathione (Hosry <i>et al.</i> , 2009a,b)		
Colloidal silver complex (CAGS) (Izquierdo-cañas <i>et al.</i> , 2012; Garcia-Ruiz <i>et al.</i> , 2015)	Inert gas (CO ₂ , Nitrogen, Argon)			

Yildirim & Darici, 2020, JMBFS

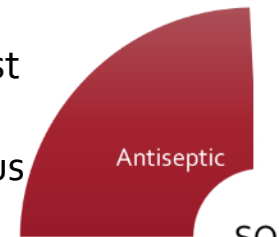
Not all these alternatives can be used in organic wines production!

SO₂ Alternatives– *post-harvest*

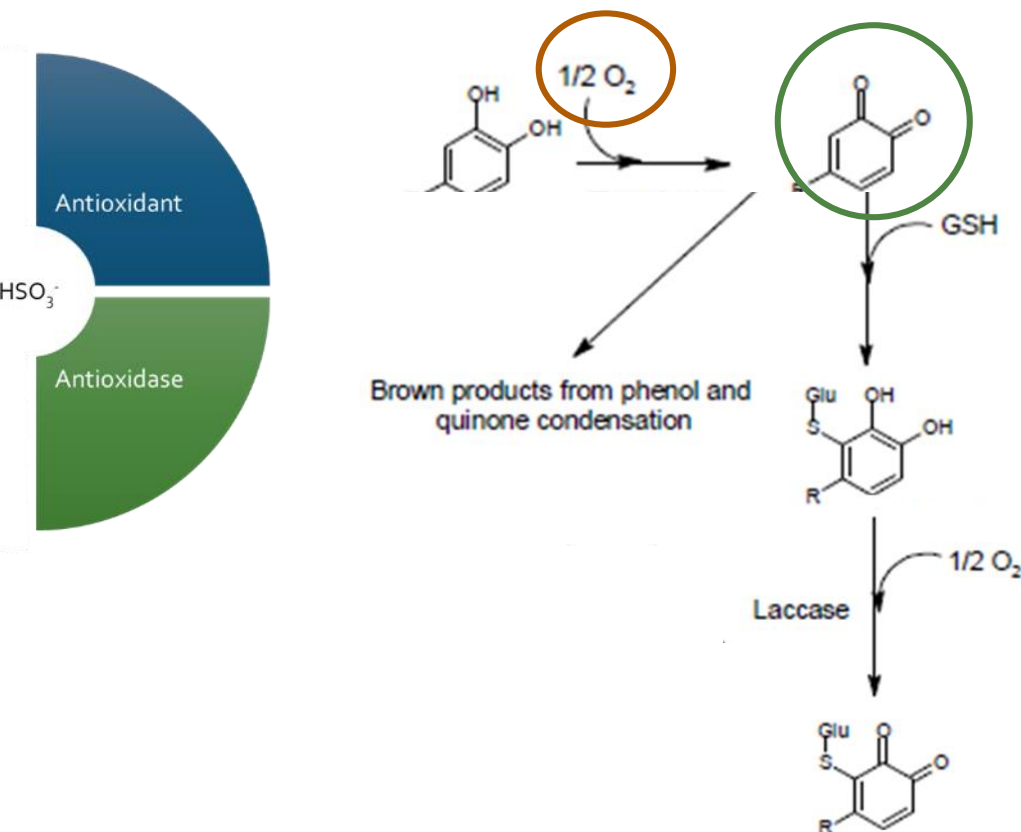


- Grape health status
- Grape conditions (machine-harvested, temperature, liquid/solid)

- Fast processing and fast fermentation start
- use ADY or spontaneous starter
- Bioprotection



SO₂



- Reducing Oxygen during crushing: **Dry Ice** and **inert gas**

- O₂ Trapping: **Ascorbic acid** (always use with SO₂)

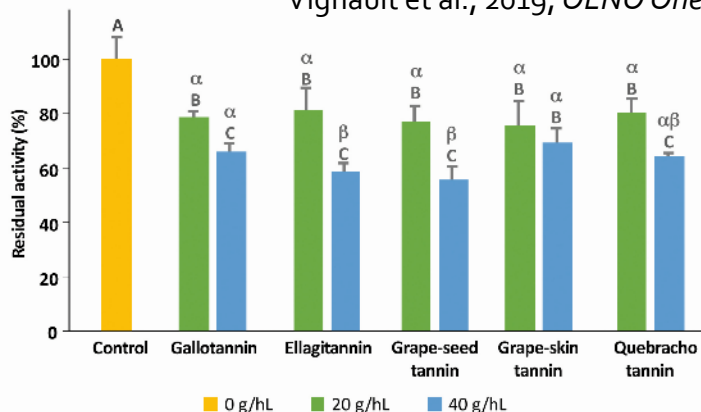
- Glutathione (not as direct addition)

- *Botrytis cinerea* laccase can oxidize glutathione adducts

SO₂ Alternatives- Focus on Oenological tannins

Inhibition of laccase activity

Vignault et al., 2019, *OENO One*



Oxygen consumption

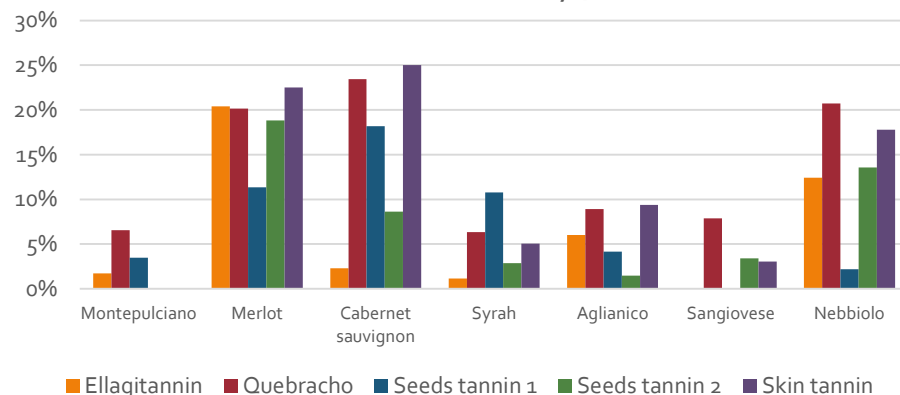
Pascual et al., 2017, *Food Chem.*

Type of tannin	OCR (mgO ₂ /day.g)		RAC (%)
Sulfur dioxide	11.90 ± 0.63	E	100
Ascorbic acid	2976 ± 625	F	50,000
Seed tannin	0.19 ± 0.05	B	13
Skin tannin	0.40 ± 0.10	C	27
Quebracho tannin	0.57 ± 0.15	C	38
Gallotannin	0.06 ± 0.05	A	4.2
Ellagitannin	1.81 ± 0.15	D	122

Color protection

Paissoni et al., 2020, *Food Chem.*

Δ% Color Intensity 72h

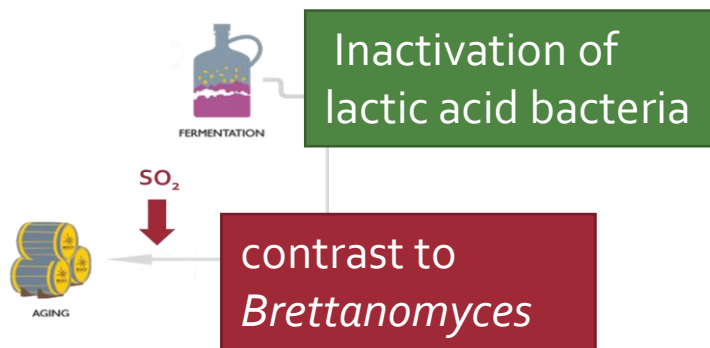


- Prevent anthocyanins oxidation
- Tannins interacts with anthocyanins: condensation and copigmentation

VARIETY EFFECT

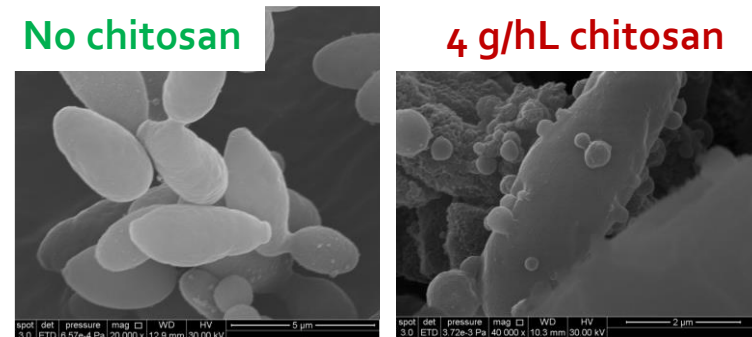
Effectiveness depends on anthocyanins ratio (different extractability and oxidability)

SO₂ Alternatives - Focus on Chitosan



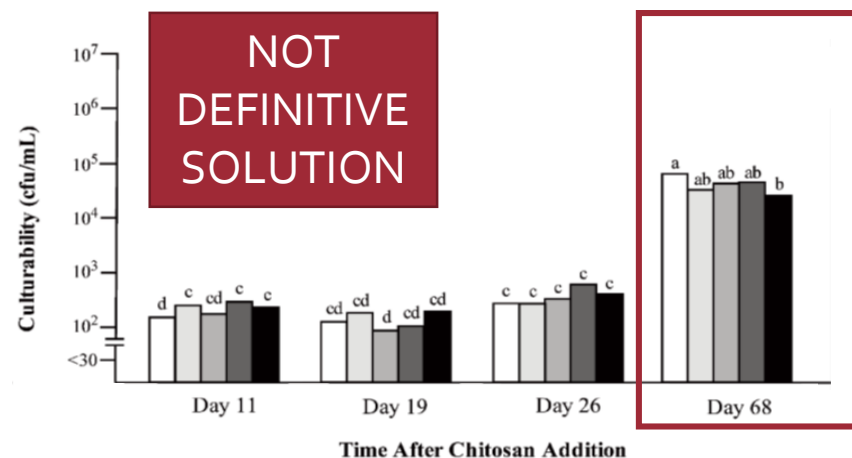
Antimicrobial activity:

Brettanomyces bruxellensis and *Oenococcus oeni*
Saccharomyces cerevisiae seemed resistant to chitosan



Chitosan was able to contain but not to completely eradicate *B. bruxellensis* from wines

Petrova et al., 2016, *OENO One*

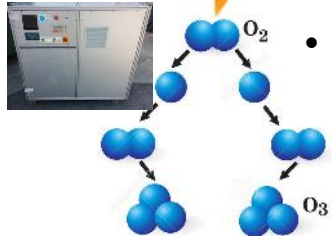


Proposed for many uses

Castro Marin et al., 2020, *Crit. Rev. Food Sci.*

- **Fining**, clarification, and protein haze prevention
- Sorption of **heavy metals**
- Removal of ochratoxin A
- **Antioxidant** activity
- Remediation of wines containing high concentrations of **volatile phenols**

Emerging technologies – Ozone (O_3)

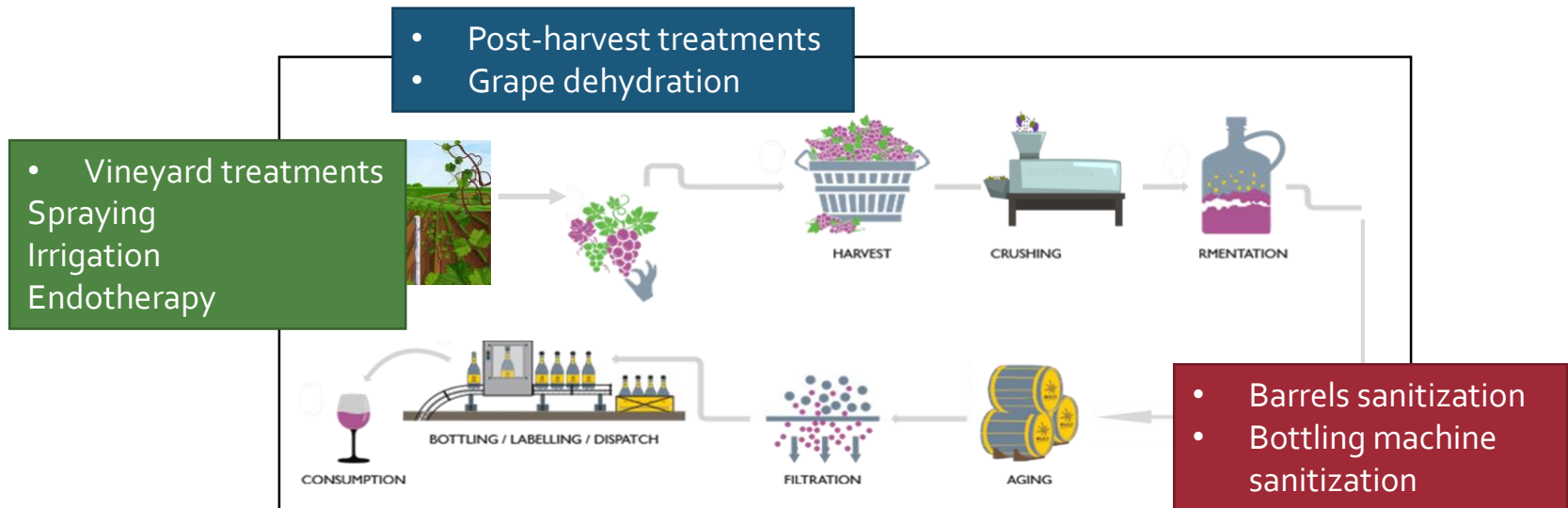


- Strong oxidant
- Strong disinfectant
- Broad action but short activity span (unstable)
- Generally safe (GRAS, no residuals on wine, but to be used with precautions)

For the moment ozone is not allowed in EU in direct contact with organic foods

USA: Ozone is allowed for processing of «organic» or «made with organic» foods (7 CFR § 205.605)

CANADA: NASAA Organic Standard allows ozone for storage (controlled atmosphere) and for washing solutions of fresh fruit.



Emerging technologies – O_3 post-harvest as antimicrobial

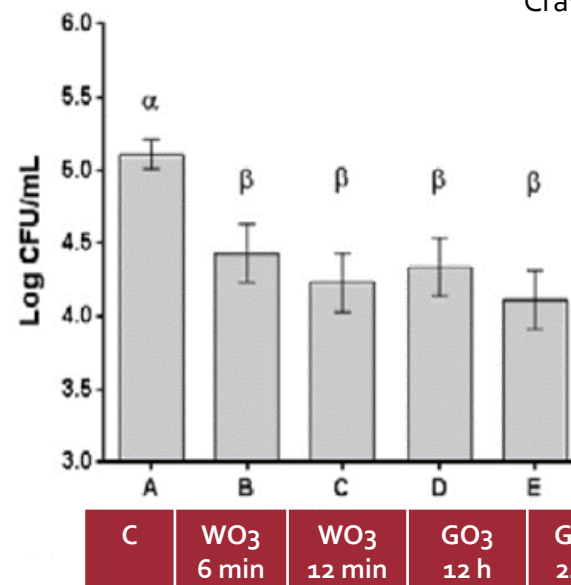
Cravero et al. 2016, *Food Res Int*



O_3

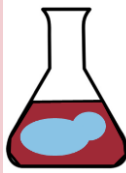


Control	untreated		
Gaseous ozone (GO_3)	32 g/h	12 hours	24 hours
Aqueous ozone (WO_3)	5 mg/L	6 minutes	12 minutes



Ozone reduced the apiculate yeasts load

Barbera O_3 treated wines

	Treatments	Acetic acid (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Residual sugars (g/L)
Spontaneous 	<i>Grape must</i>	<0.1	0.1 ± 0.1	<0.1	216.6 ± 10.1
	SA	0.52 ± 0.05bc	10.2 ± 0.7	12.6 ± 0.1	<2.0
	SB	0.16 ± 0.05a	10.3 ± 0.9	12.3 ± 0.3	<2.0
	SC	0.20 ± 0.01 a	9.9 ± 0.4	12.3 ± 0.2	<2.0
	SD	0.38 ± 0.02ab	9.9 ± 0.2	12.5 ± 0.2	<2.0
	SE	0.35 ± 0.08ab	10.2 ± 0.1	12.6 ± 0.1	<2.0
Inoculated 	IA	0.28 ± 0.09a	10.5 ± 0.1	12.8 ± 0.3	<2.0
	IB	0.25 ± 0.01 a	10.1 ± 0.2	12.7 ± 0.1	<2.0
	IC	0.25 ± 0.05a	10.6 ± 0.4	12.7 ± 0.5	<2.0
	ID	0.31 ± 0.08a	9.3 ± 0.8	12.7 ± 0.1	<2.0
	IE	0.27 ± 0.03a	10.2 ± 0.5	12.8 ± 0.3	<2.0
	Sign.	**	NS	NS	NS

Ozone reduced the acetic acid production in spontaneous fermentation



At the moment ozone is not allowed in EU in direct contact with organic foods

Emerging technologies - O_3 *Brettanomyces*

Barbera in perforated boxes (2Kg) was inoculated with *B. bruxellensis*

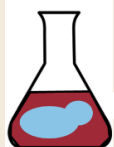
Cravero et al. 2018, IFSET



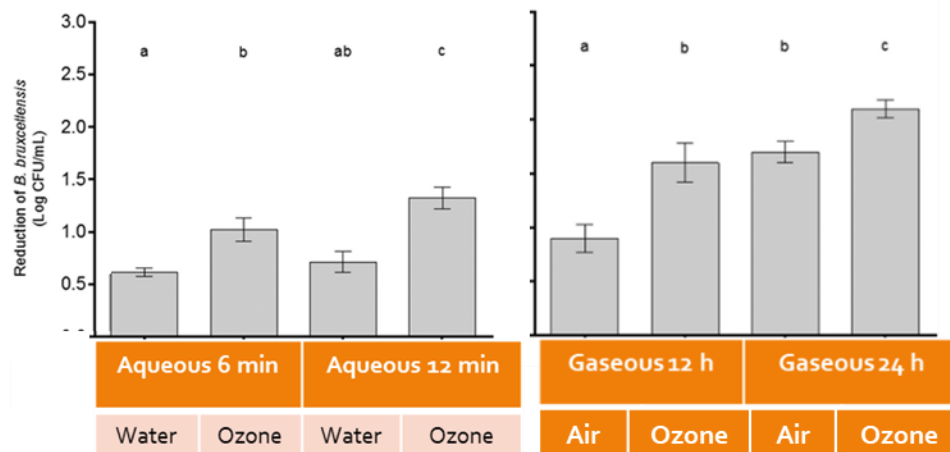
O_3



Gaseous ozone (GO_3)	32 g/h	12 hours	24 hours
Aqueous ozone (WO_3)	5 mg/L	6 minutes	12 minutes



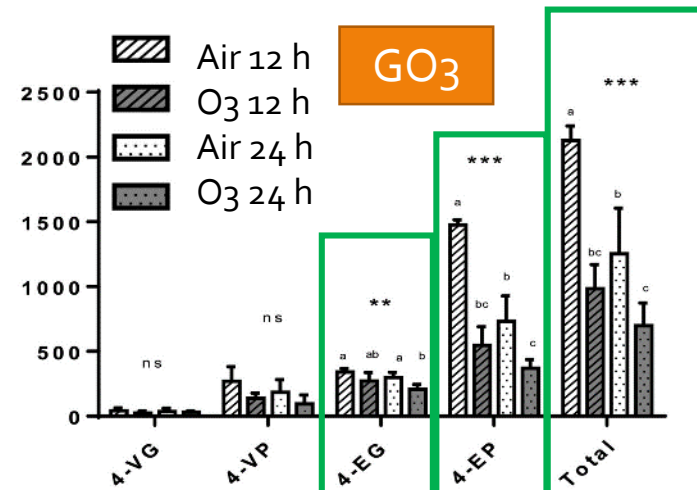
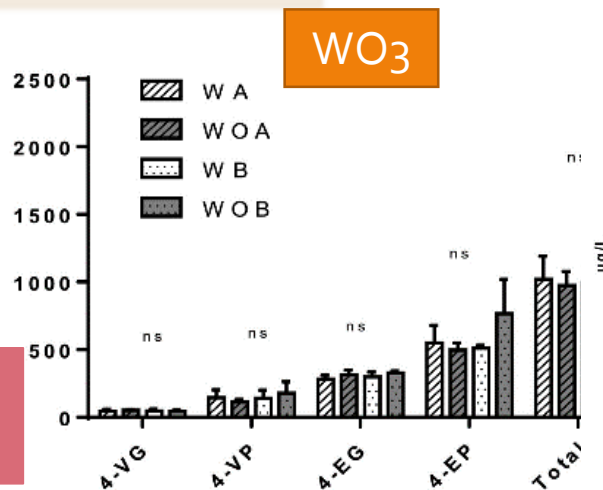
HS-SPME-GC-MS
analysis of volatile phenols



Ozone reduction of *Brettanomyces* led to significant decrease of ethyl phenols in wine



At the moment ozone is not allowed in EU in direct contact with organic foods

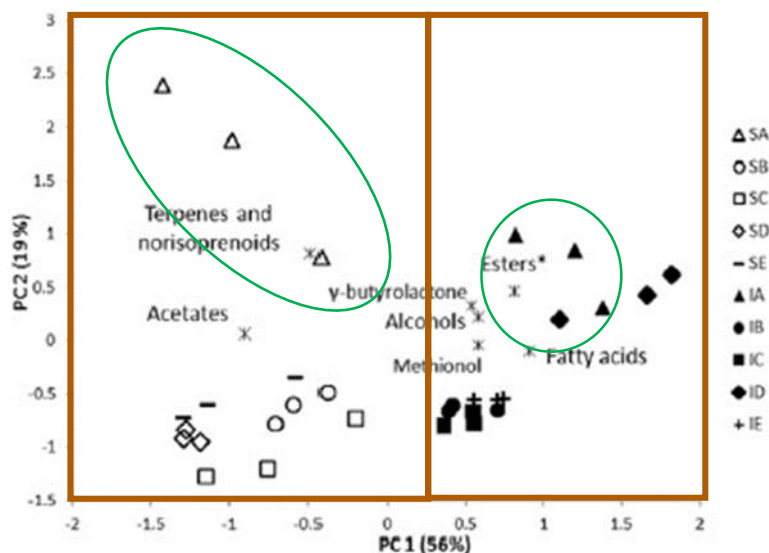


Emerging technologies - O₃ Aroma

Does O₃ modify grape and wine quality parameters?

GO₃ and AO₃ Post-harvest treatment (cv Barbera)

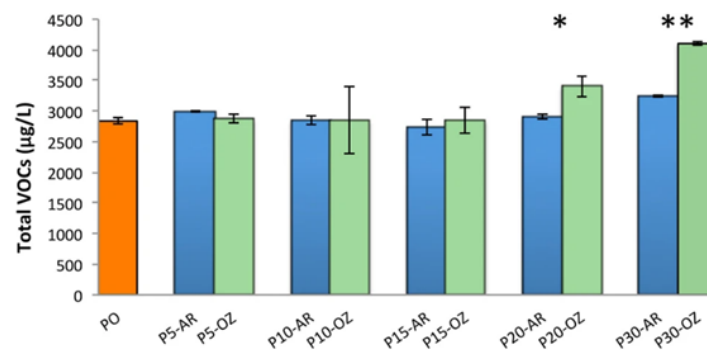
Cravero et al. 2016,
Food Res Int



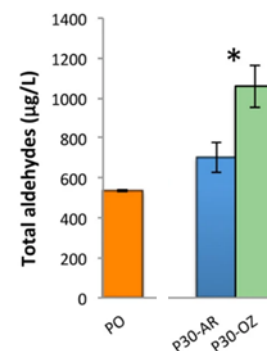
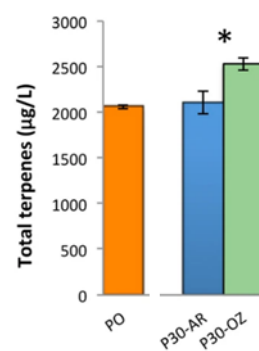
GO₃ (30 uL) grape dehydration (cv Moscato)

Rio Segade et al. 2017 *SciRep*

1° experience on other grape



Increase in terpenoids and C-6 compounds



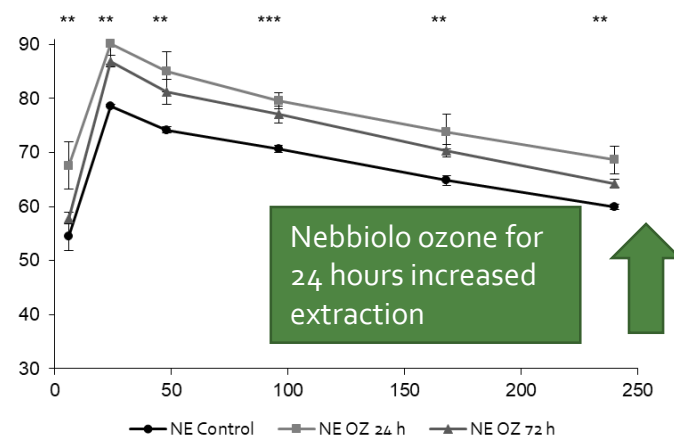
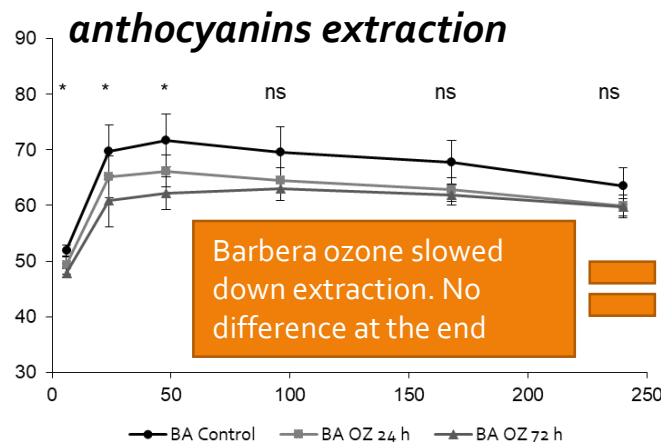
Biosynthesis of **monoterpenes** and of **aldehydes**

Confirmation at molecular level of the **elicitor activity of ozone**

Emerging technologies - O₃ Phenolics

Paissoni et al., 2017,
Food Res Int

GO₃ Post-harvest
treatment (cv Barbera
and Nebbiolo)



flavan-3-ols extraction

In Barbera no differences
among treatments

In Nebbiolo: OZONE
increased the flavan-3-
ols extracted

Anthocyanins
protection?

GO₃ Dehydration (cv
Barbera and Nebbiolo)

Rio Segade et al.,
2019, *Food Chem*;
Rio Segade et al.
2020, *JAFAC*

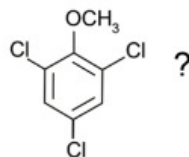
			Fresh berries	10% Average berry WL			20% Average berry WL			Variety effect
				Air	Ozone	Sign	Air	Ozone	Sign	
Barbera	TA	mg /kg grape	1534±95	1491±7	1558±58	ns	1487±170	1441±75	ns	↓
	FRV	mg / kg grape	397±43	486±63	479±56	ns	603±107	512±20	ns	
	PRO	mg /kg grape	1495±111	1696±165	1825±96	ns	1868±136	1607±64	*	
	mDP		9.99±0.08	10.46±0.2	12.61±0.4	*	15.74±0.46	11.76±0.18	***	
Nebbiolo	TA	mg /kg grape	612±36	701±40	710±12	ns	760±25	796±29	ns	↑
	FRV	mg / kg grape	1658±99	1907±178	1809±129	ns	2084±200	2389±33	*	
	PRO	mg /kg grape	3607±225	4419±138	4026±320	ns	5123±453	5003±456	ns	
	mDP		16.55±0.04	18.69±0.67	16.96±0.38	*	17.02±0.26	17.72±0.90	ns	

Emerging technologies – EW: another option?

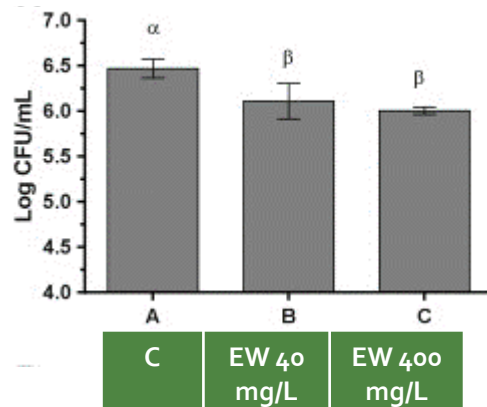
Electrolized water (EW)
is a sanitization agent

EW is produced by electrolysis
of dilute solutions of NaCl (also
KCl or MgCl₂)

Hypochlorite ions
(ClO⁻; 80–95%)
residual on
surface



Grape
microbial load
reduction



Cravero et al., 2016, IFSET

Barbera EW treated wines

Treatment	Residual sugars (g/L)	Acetic acid (g/L)
Must	216.6 ± 10.1	nd
Control SA	1.0 ± 0.5b	0.52 ± 0.04a
Treatment SB	0.4 ± 0.0a	0.33 ± 0.16b
Treatment SC	0.4 ± 0.0a	0.21 ± 0.00b
Control IA	0.7 ± 0.2ab	0.28 ± 0.07b
Treatment IB	0.7 ± 0.1ab	0.24 ± 0.01b
Treatment IC	0.8 ± 0.0b	0.21 ± 0.02b
Sign. ^a	**	***

Issue: EW and chlorine residues Giacosa et al., 2019, Food Res Int

Effect of short-term grape storage and grape withering time in the sunlight after electrolyzed water solution treatment on the production of chloroanisoles and chlorophenols in wines after four months from the end of alcoholic fermentation.

Wine code	Grape storage time	[TCA]		[PeCA]		[TCP]	
		ng/L wine		ng/L wine		ng/L wine	
EW Post-harvest treatment	Control (no EW treatment, no storage)	nd	a	nd	a	nd	a
	Treated with EW and then rinsed with water (0 h)	2.6 ± 1.1	b	13.4 ± 6.4	a	62.4 ± 3.7	b
	4 h	3.7 ± 0.2	b	12.3 ± 6.6	a	76.0 ± 19.8	b
	24 h	2.2 ± 0.1	ab	4.9 ± 1.0	a	70.0 ± 5.7	b
	Sign.	*		ns		**	
EW Post-harvest treatment + dehydration	Control (no EW treatment, no storage)	nd	a	nd	a	nd	a
	96 h	1.6 ± 0.4	ab	7.4 ± 2.8	a	67.5 ± 31.8	a
	168 h	1.0 ± 0.6	ab	9.1 ± 3.5	a	61.5 ± 16.3	a
	500 h	2.3 ± 0.5	b	16.5 ± 3.5	a	38.0 ± 11.3	a
	Sign.	*		ns		ns	

Chloroanisols
formation

EW postharvest
treatments are
NOT SUITABLE
for wine
production

Conclusions

Red winemaking
without SO₂
addition is
possible

- Healthy grapes
- Control of fermentation
- Control of oxidation factors

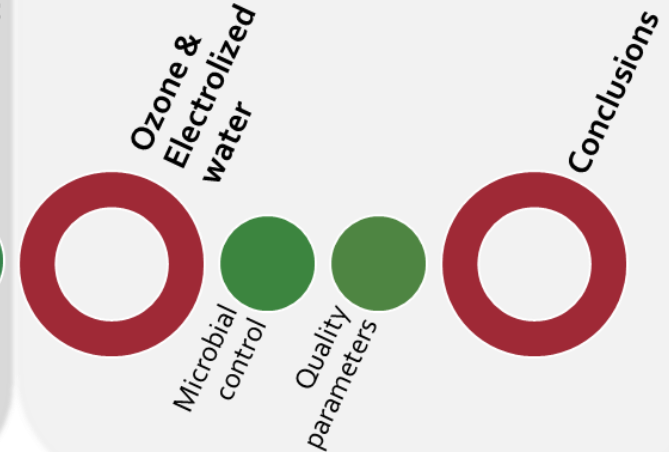
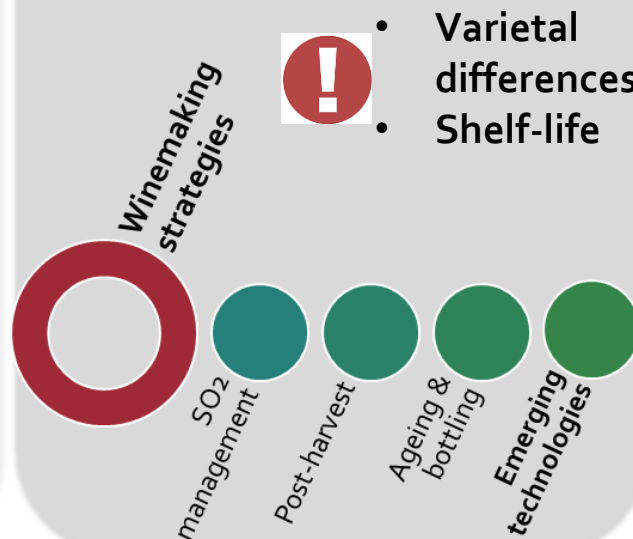
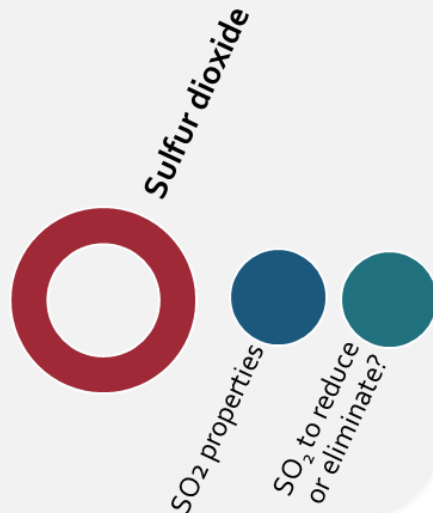
Alternatives options are
available in organic winemaking
Oenological tannins
Chitosan

OZONE treatments on grapes
may be a promising **tool** in
organic winemaking since it is
antiseptic, does **not leave**
residuals, and does **not**
negatively affect quality
parameters



At the moment ozone is not allowed in EU in direct contact with organic foods

EW: risk of chloroanisoles





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Thank you for your attention! Questions?

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