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

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Summary

Sulfur dioxide
- SO_2 properties
- SO_2 to reduce or eliminate it?

Winemaking strategies
- SO_2 management
- Post-harvest
- Ageing & bottling
- **Emerging technologies**

Potential Applications: ozone & electrolized water
- Microbial control
- Quality parameters

Conclusions

Sulfur dioxide - *properties*

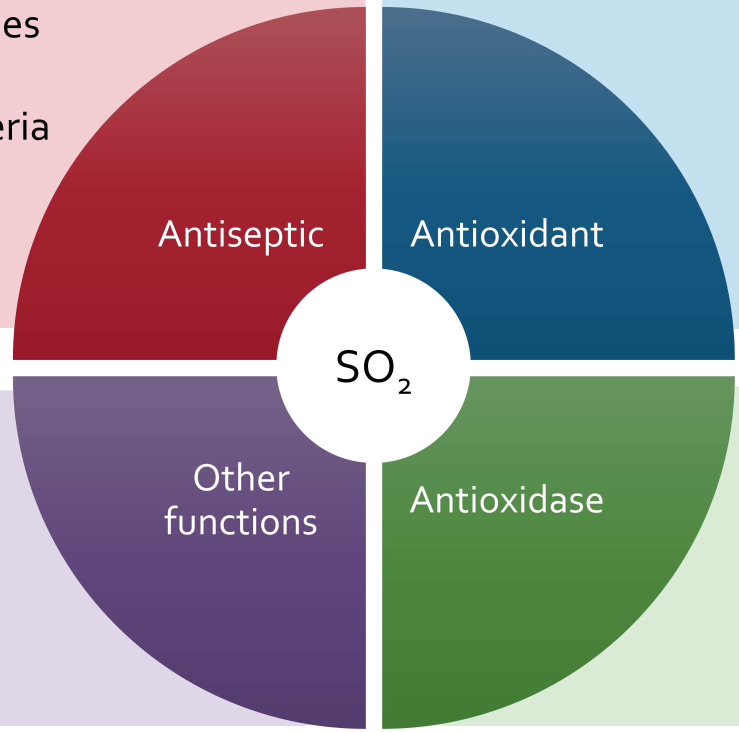


SO_2 selects the must microflora and acts as an antimicrobial in wines
- apiculate yeasts
- acetic and lactic acid bacteria
- *Brettanomyces* spp.

Chemical oxidation
Polyphenols
Oxygen
Metals
Radical species

Acetaldehyde

Factors



- Solubilizing agent
- Fining agent
- Binding agent

Enzymatic oxidation
Grape PPO:
Tyrosinase

Botrytis cinerea PPO:
Laccase




Sulfur dioxide– *To reduce or eliminate it?*

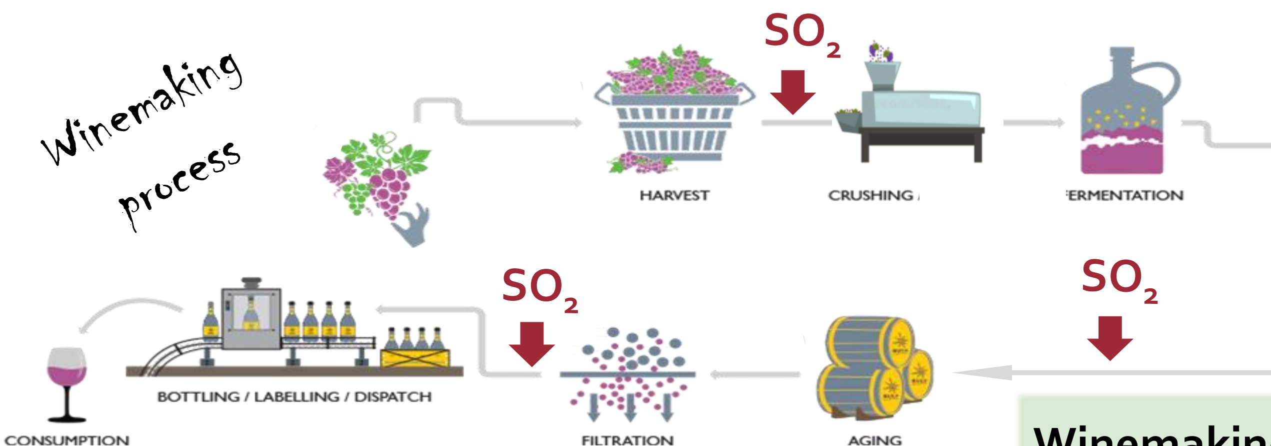
- Easy to use
- Easy to produce
- Cheap
- Effective

Health issues

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

Avoid SO_2 to improve the "naturalness" of wines

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



EU SO_2 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*

Antiseptic

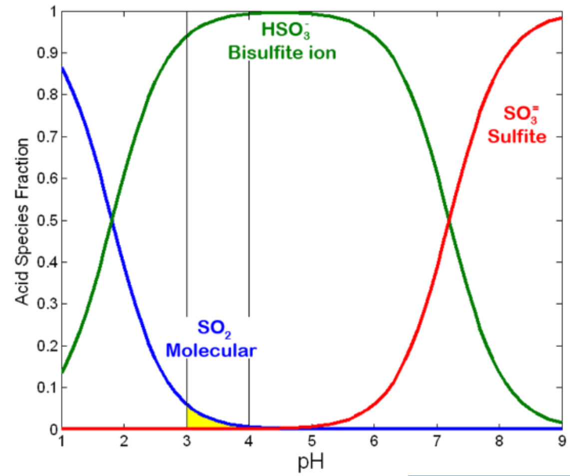
SO_2

Molecular SO2 Factors:

- ↑ pH ↓
- ↑ ethanol ↑
- ↑ temperature ↑

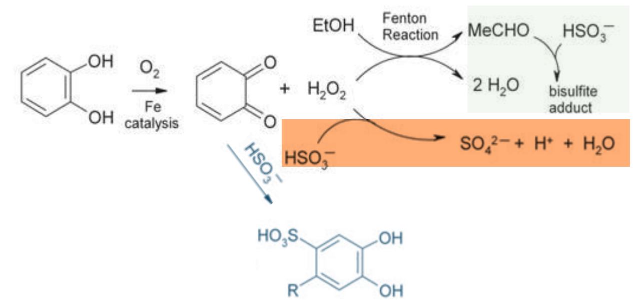
Bacteria : 0.5- 1 mg/L
Yeasts: 1 - 2 mg/L

$$SO_2(gas) \leftrightarrow \boxed{SO_2(aq) \xleftrightarrow{pK_{a1} \approx 1.8} HSO_3^-(aq)} \xleftrightarrow{pK_{a2} \approx 7} SO_3^{2-}(aq)$$



Antioxidant

HSO_3^-



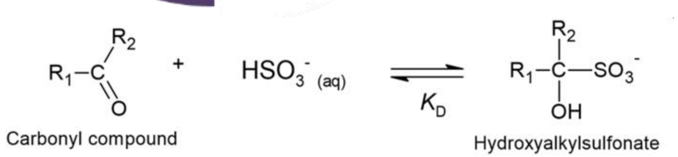
HSO_3^-

Antioxidase

Other functions

HSO_3^-

Binding
Acetaldehyde irreversible
Other acid and polyphenols reversible



Target	
$SO_2(aq) \leftrightarrow HSO_3^-(aq)$ **Free SO2** ↑	Bound SO2 ↓

SO_2 Alternatives

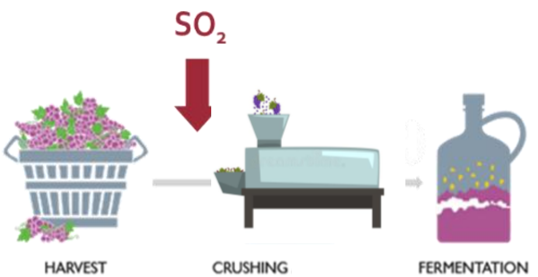
Table 2 Some alternatives of SO_2 used during wine production

A. Chemical Additives	B. Non-thermal Applications	C. Phenolic Compounds and Plant Extracts	D. Killer Toxins and Bacteriocins	E. Combine Metnods
DMDC (Costa and Loureiro, 2008)	High Hydrostatic Pressure (HHP) (Briones-Labarca *et al.*, 2017)	Eucalyptus and almond skin extracts (Garcia-Ruiz *et al.*, 2013b)	*Kluyveromyces phaffii* DBCG 6076 (Ciani *et al.*, 2001)	Glutathione and cafeic acid or gallic acid (Roussis and Sergianitis, 2008)
Lysozyme (Azzolini *et al.*, 2010)	Pulsed Electric Field (PEF) (Delsart *et al.*, 2015a,b)	Stilbens extracts (Raposo *et al.*, 2016b)	Pediocin PA-1 (Diez *et al.*, 2012)	Glutathione and/ or elligatannis (Panero *et al.*, 2015)
Ascorbic acid (Sonni *et al.*, 2011)	Ultraviolet Irradiation (UV-C) (Fredericks and Krügel 2011)	Thyme essential oil (Freidman *et al.*, 2017)	Nisin (Rojo-bezares *et al.*, 2007)	Glutathione and ascorbic acid(Comuzzo *et al.*, 2015)
Ethanethiol (Dias *et al.*, 2013)	Ultrasound (HPU) (Jiranek *et al.*, 2008; Gracin *et al.*, 2016)	Grape and wood tannins (Sonni *et al.*, 2009; Alamo-Sanza *et al.*, 2019;Sánchez-Palomo *et al.*, 2017)	Killer toxins CpKT1 andCpKT2 (Mehlomakulu *et al.*, 2014)	Lysozyme and polyphenols (Chen *et al.*, 2015)
NaOCl (sodium hypochlorite) (Yoo *et al.*, 2011)	Dry Ice Application (Costantini *et al.*, 2015)	Hydroxytyrosol and oleuropein (Raposo *et al.*, 2016a,b)	Lacticin 3147 (Garcia-Ruiz *et al.*, 2013a)	Lysozyme and tannins (Sonni *et al.* ,2009)
Chitosan (Chinnici *et al.*, 2014; Elmacı *et al.*, 2015)		Glutathione (Hosry *et al.*,2009a,b)		
Collaidal silver complex (CAgS) (Izquierdo-cañas *et al.*, 2012; Garcia-Ruiz *et al.*, 2015)	Inert gas (CO2, Nitrogen, Argon)			

Yildirim & Darici, 2020, JMBFS

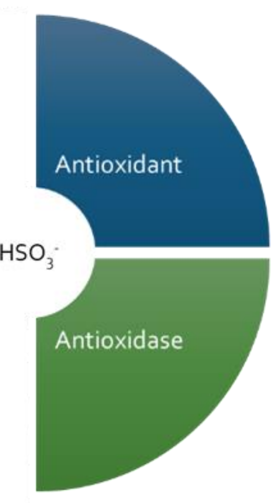
Not all these alternatives can be used in organic wines production!

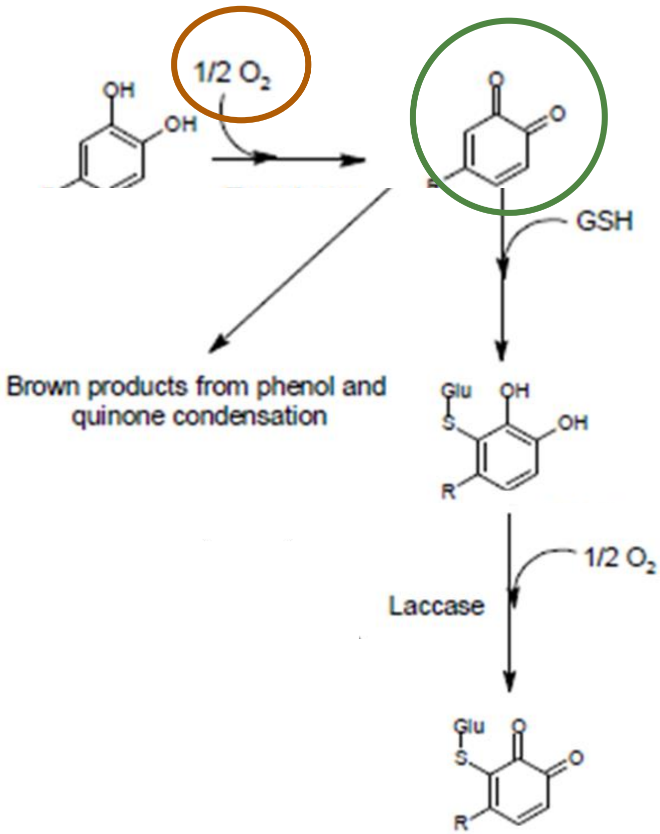
SO_2 Alternatives– *post-harvest*



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

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





- Reducing Oxygen during crushing: **Dry Ice** and **inert gas**
- O2 Trapping: **Ascorbic acid** (always use with SO2)

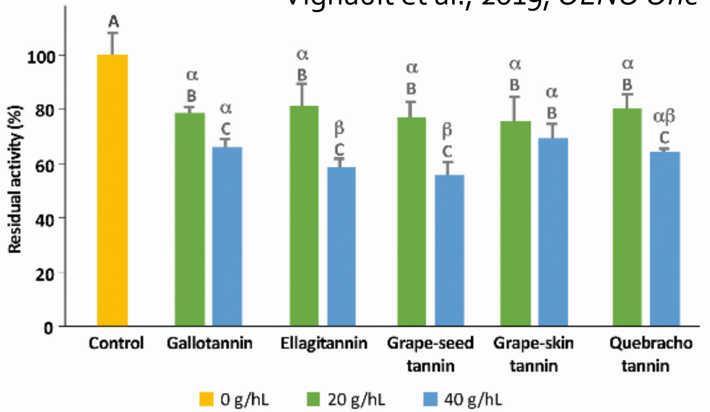
- Glutathione (not as direct addition)

- *Botrytis cinerea* laccase can oxidize glutathione adducts

SO_2 Alternatives- *Focus on Oenological tannins*

Inhibition of laccase activity

Vignault et al., 2019, *OENO One*



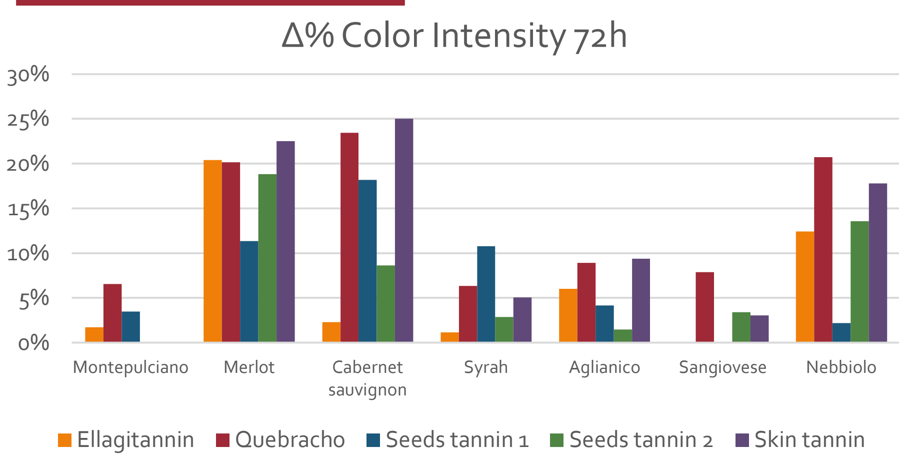
Oxygen consumption

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

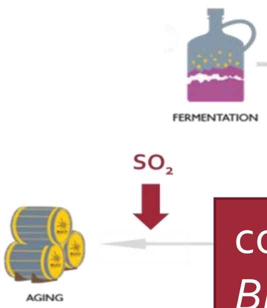
Paissoni et al., 2020, *Food Chem.*



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

VARIETY EFFECT
Effectiveness depends on anthocyanins ratio (different extractability and oxidability)

SO_2 Alternatives - *Focus on Chitosan*



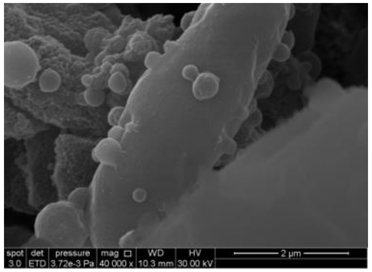
Inactivation of lactic acid bacteria

contrast to *Brettanomyces*

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

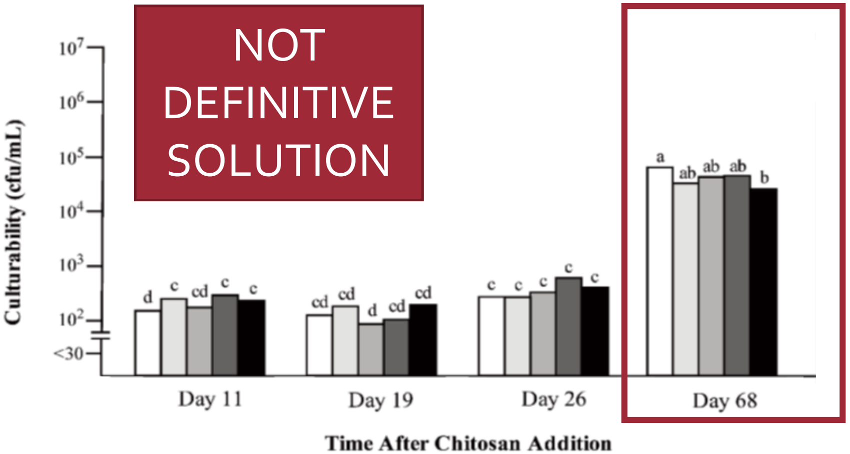
No chitosan

4 g/hL chitosan



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

Petrova et al., 2016, *OENO One*

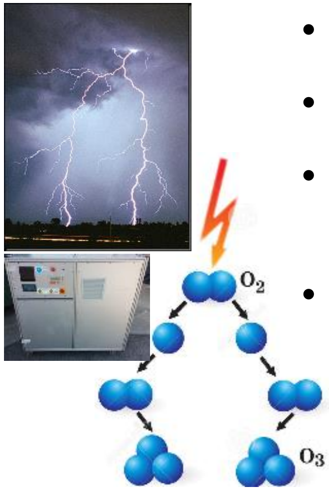


Proposed for many uses

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)

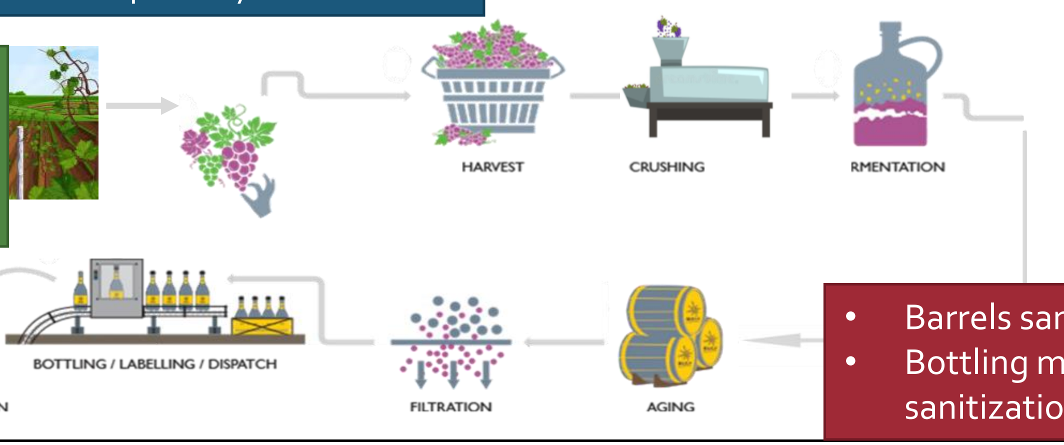
For the moment ozone is <u>not allowed in EU</u> in direct contact with organic foods

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.

- Post-harvest treatments
- Grape dehydration

- Vineyard treatments
Spraying
Irrigation
Endotherapy



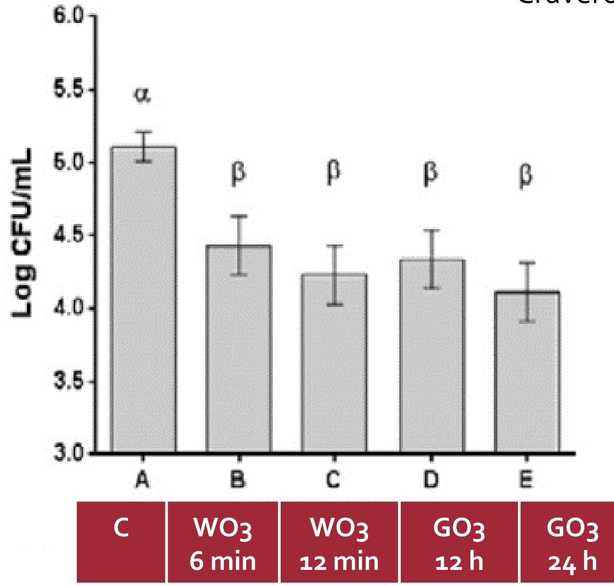
- Barrels sanitization
- Bottling machine sanitization

Emerging technologies – *O_3 post-harvest as antimicrobial*

Cravero et al. 2016, *Food Res Int*

O_3

Control	untreated		
Gaseous ozone (GO3)	32 g/h	12 hours	24 hours
Aqueous ozone (WO3)	5 mg/L	6 minutes	12 minutes

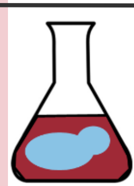


Ozone reduced the apiculate yeasts load

Barbera O3 treated wines

Spontaneous

Inoculated



Treatments	Acetic acid (g/L)	Glycerol (g/L)	Ethanol (% v/v)	Residual sugars (g/L)
Grape must	<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
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

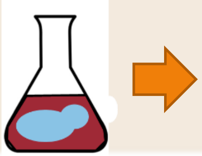
At the moment ozone is <u>not allowed in EU</u> in direct contact with organic foods

Emerging technologies - O_3 *Brettanomyces*

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

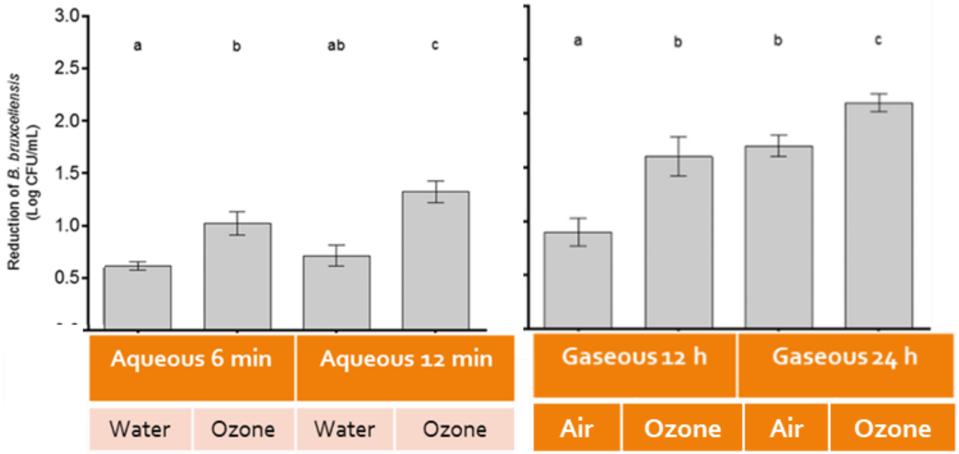
O_3

Gaseous ozone (GO3)	**32 g/h**	**12 hours**	**24 hours**
Aqueous ozone (WO3)	5 mg/L	6 minutes	12 minutes

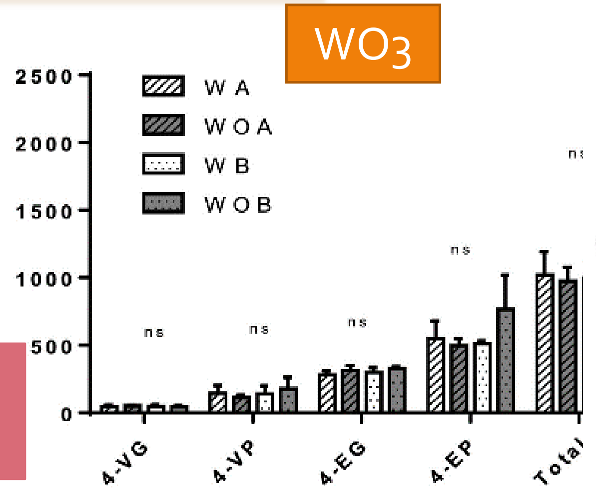


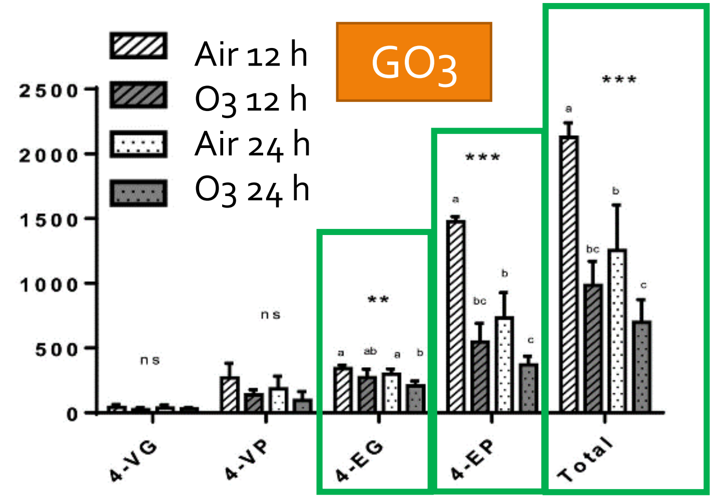
HS-SPME-GC-MS
analysis of volatile phenols

Cravero et al. 2018, IFSET



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





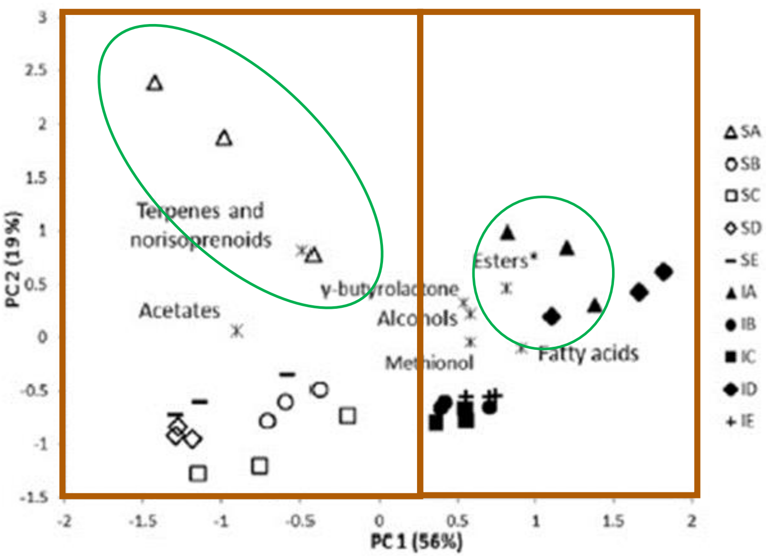
At the moment ozone is not allowed in EU in direct contact with organic foods

Emerging technologies - O_3 *Aroma*

Does O3 modify grape and wine quality parameters?

GO3 and AO3 Post-harvest treatment (cv Barbera)

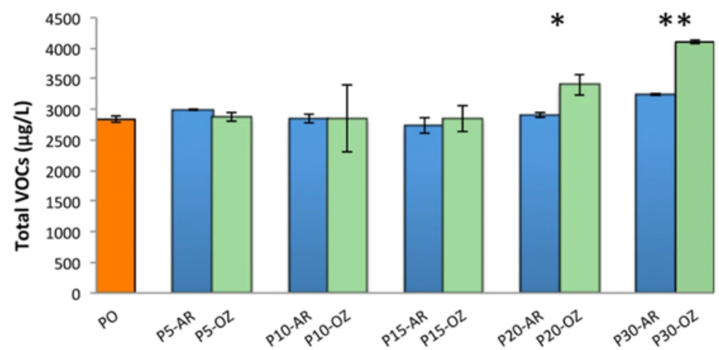
Cravero et al. 2016, *Food Res Int*



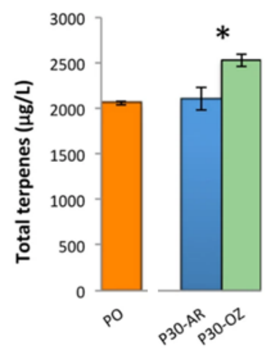
GO3 (30 uL) grape dehydration (cv Moscato)

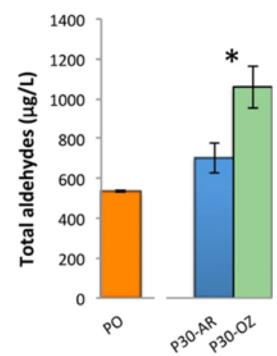
Rio Segade et al. 2017 *SciRep*

1° experience on other grape



Increase in terpenoids and C-6 compounds





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

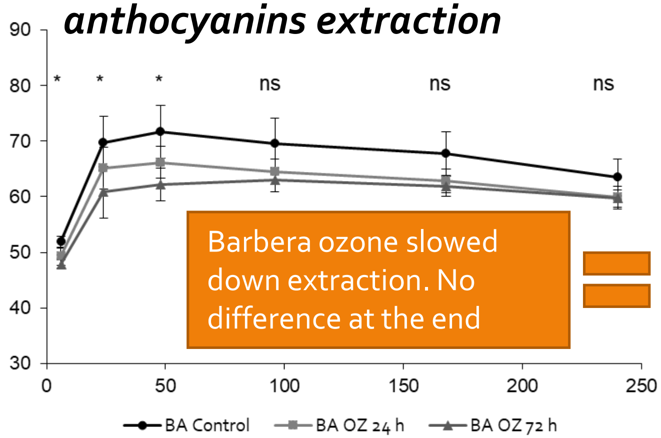
Confirmation at molecular level of the **elicitor activity of ozone**

Emerging technologies - O_3 *Phenolics*

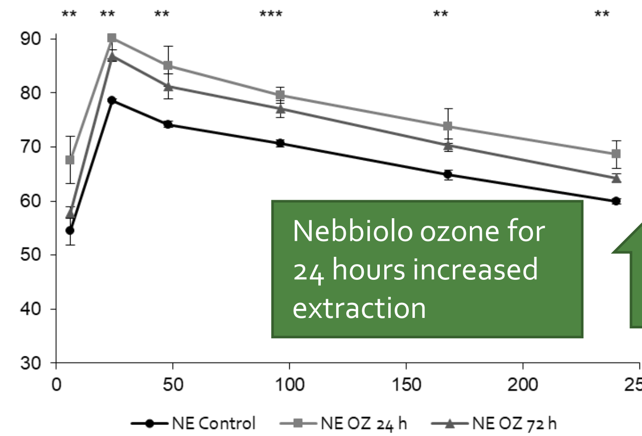
Paissoni et al., 2017, *Food Res Int*

GO3 Post-harvest treatment (cv Barbera and Nebbiolo)

anthocyanins extraction



Barbera ozone slowed down extraction. No difference at the end



Nebbiolo ozone for 24 hours increased extraction

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, *JAFC*

			Fresh berries	10% Average berry WL			20% Average berry WL		
				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

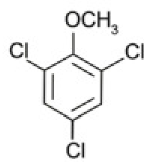
Variety effect

Emerging technologies – *EW: another option?*

Electrolized water (EW) is a sanitization agent

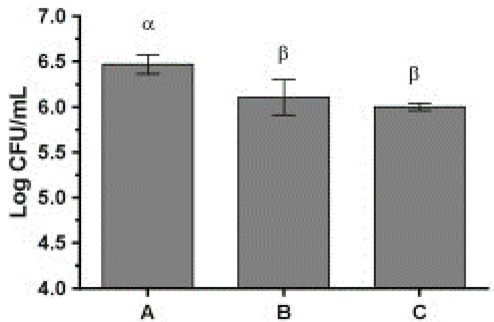
EW is produced by electrolysis of dilute solutions of NaCl (also KCl or $MgCl_2$)

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



?

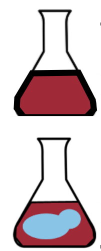
Grape microbial load reduction



C	EW 40 mg/L	EW 400 mg/L

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	*rage*						
	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

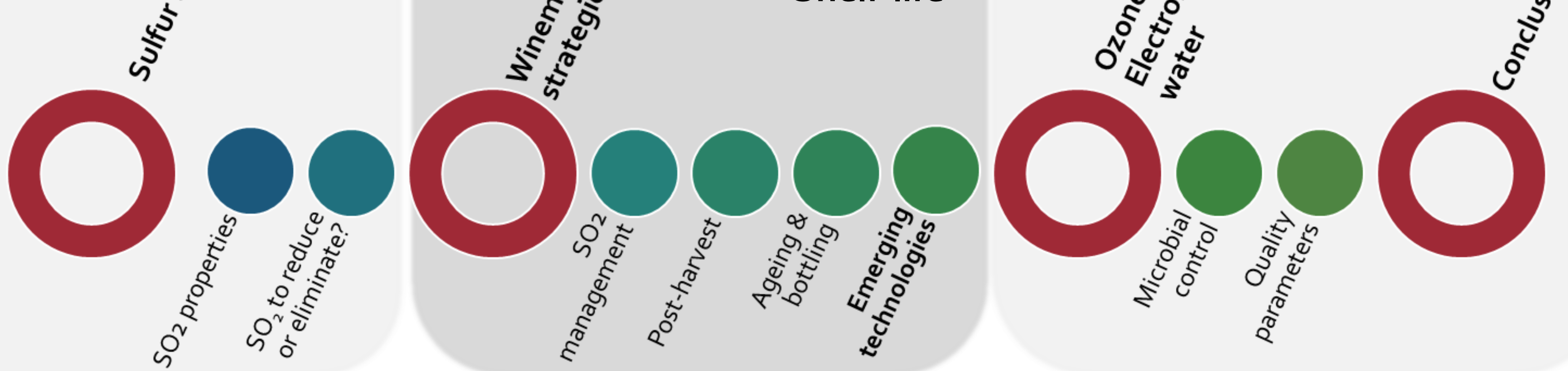
Chitosan

- **Varietal differences**
- **Shelf-life**

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 <u>not allowed in EU</u> in direct contact with organic foods

EW: risk of chloroanisoles



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Thank you for your attention!
Questions?

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