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Proctase and other alternatives

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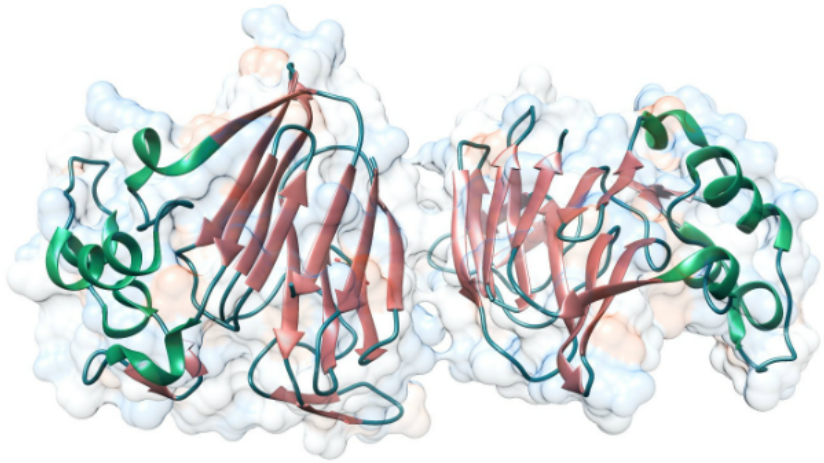
How can we used our knowledge to prevent haze formation?

- Positively charged at wine pH
- High stability at low pH
- Robust proteins (globular)
- High resistance to proteolysis
- Unfolding mostly heat-mediated
- Tendency to get insoluble during wine storage



VVTL1 - 4L5H

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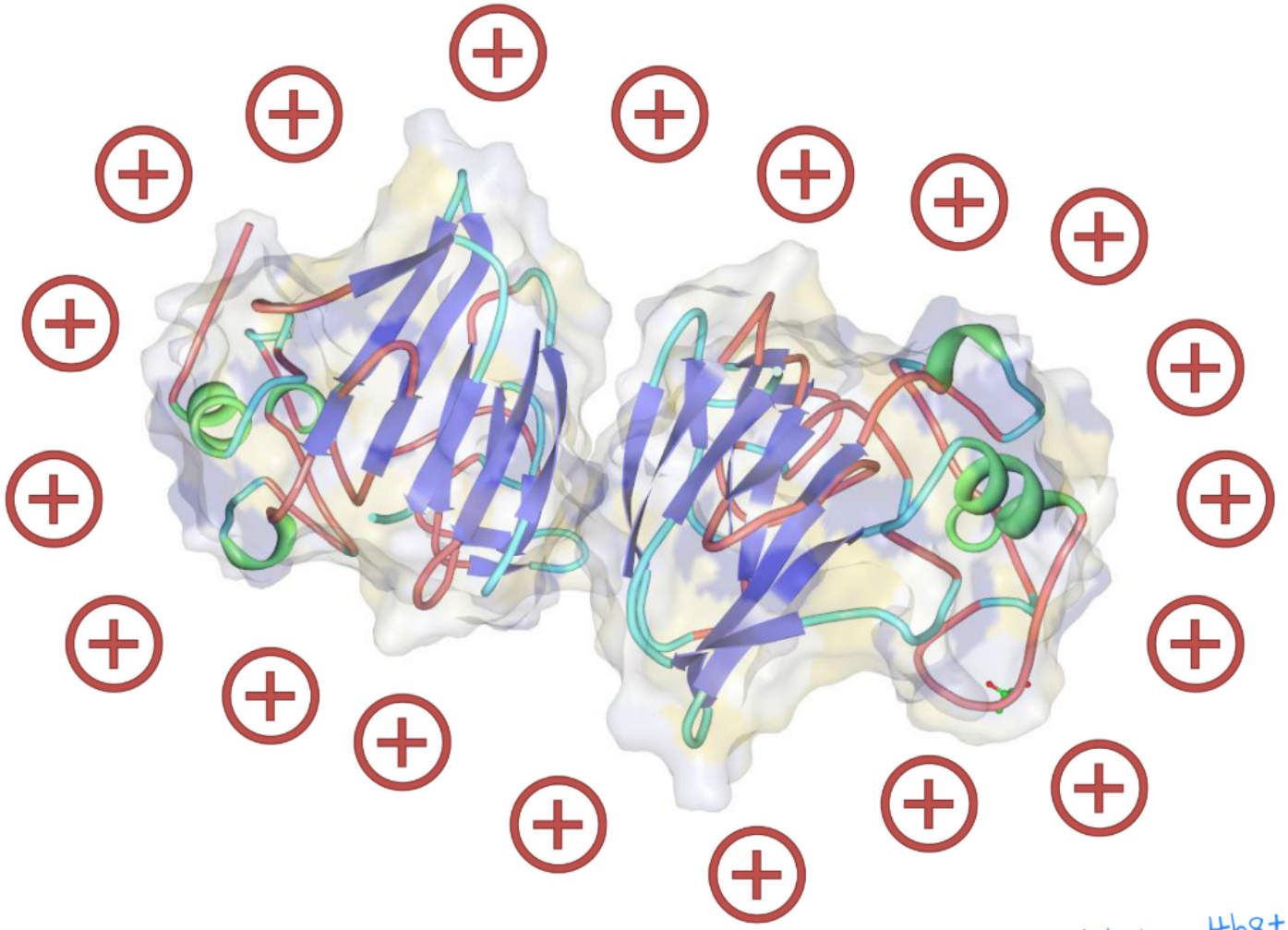
Positively Charged



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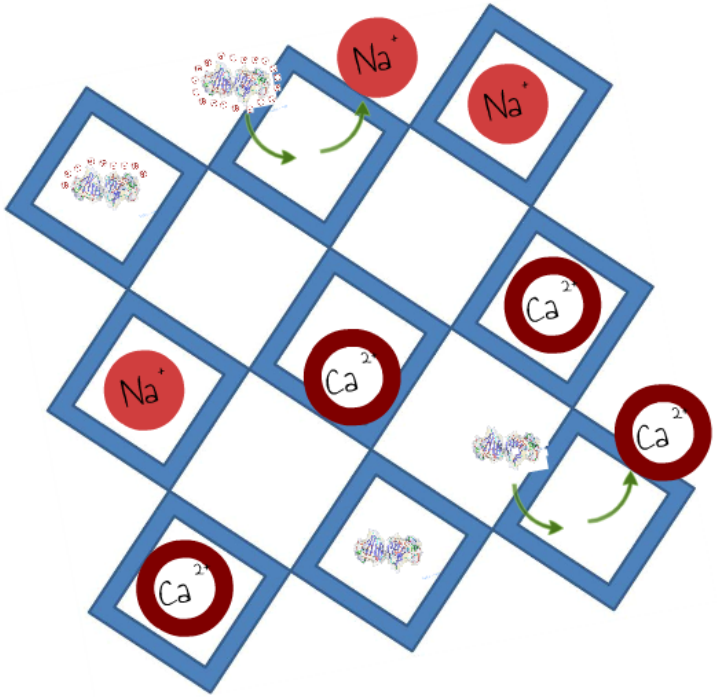
Current solution



Bentonite is a clay cation exchanger that binds proteins and loosely settles to the bottom of wine tanks

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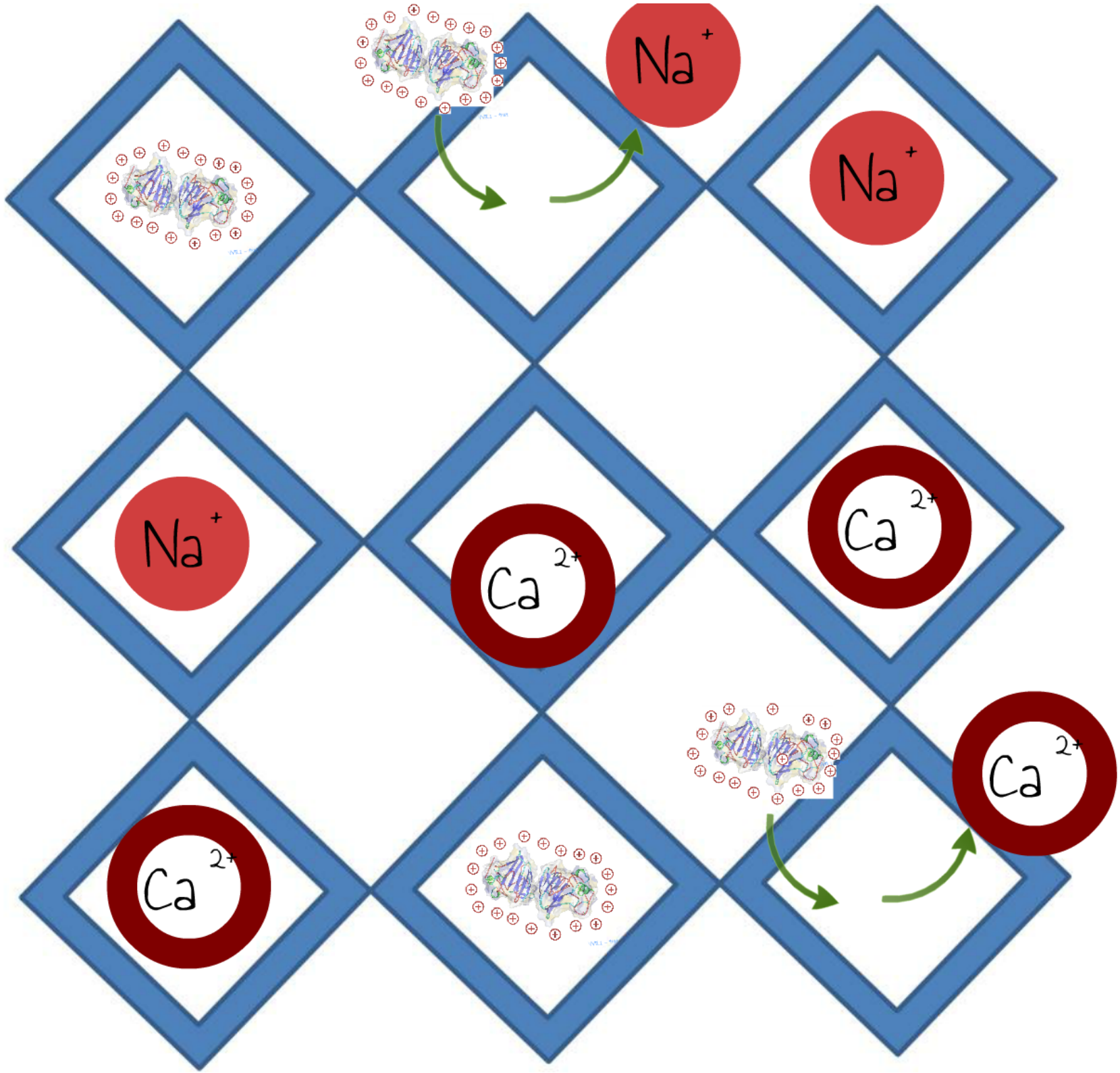

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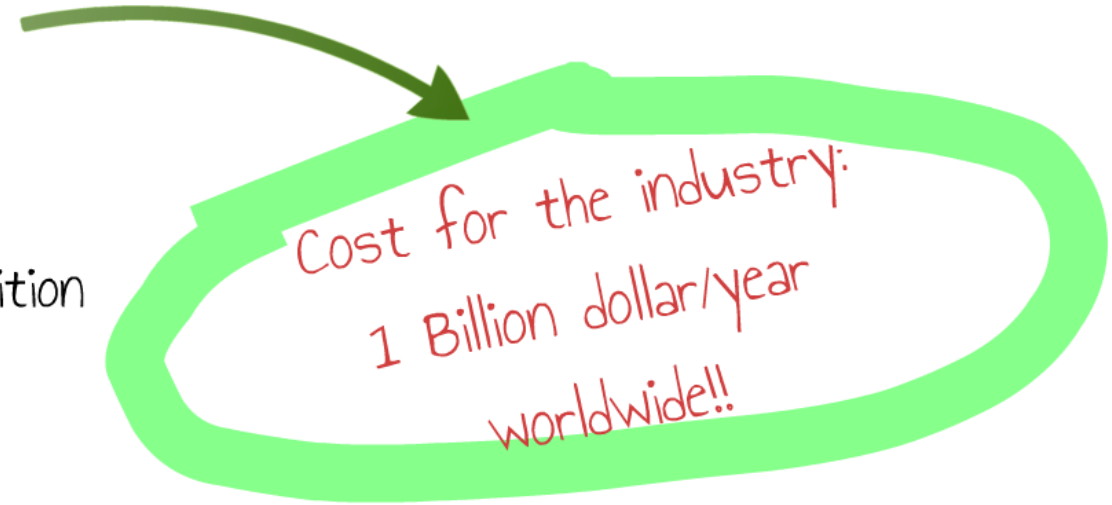
Disadvantages:

- Loss of wine (3%) as lees
- waste disposal costs
- lack of specificity for protein
- logistics of preparation and addition
- OH&S issues for its handling
- water addition: 3% v/v limit



Majewski, P.; Barbalet, A.; Waters, E. J. Grapegrower & Winemaker 2011, 58-62.

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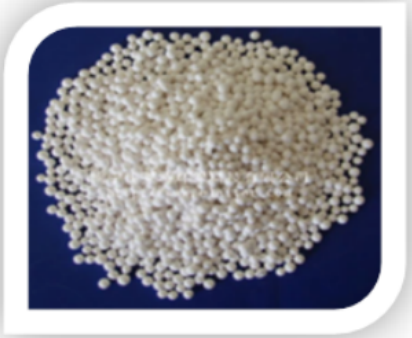
Alternatives to bentonite proposed

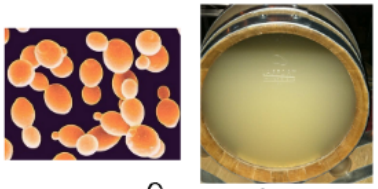
Chitinases adsorption on column of chitin

(Vincenzi et al., 2005)



Adsorbent resins (Dowex, Amberlite, ...)

(Gump and Huang, 1999)



Haze protective factor & mannoproteins

(Schmidt et al, 2010)

Zirconia dioxide

(Pashova et al., 2004)

Our focus on:

ZIRCONIA, CARRAGEENAN, PROTEASES

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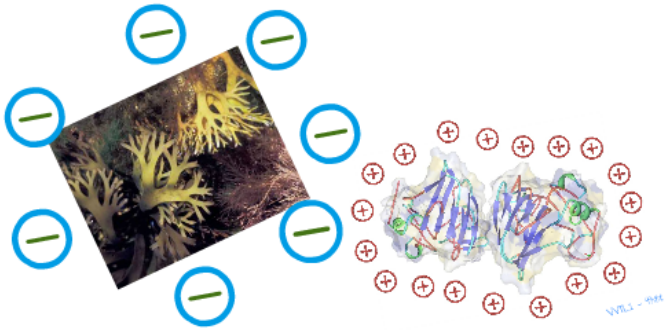
Carrageenan

Carrageenan is a cell wall hydrocolloyd found in certain species of red seaweeds

Used in beer for protein stabilization and copper fining



Seaweeds farming

Proven to be effective in removing grape and wine proteins with low sensory and chemical impacts on treated wines

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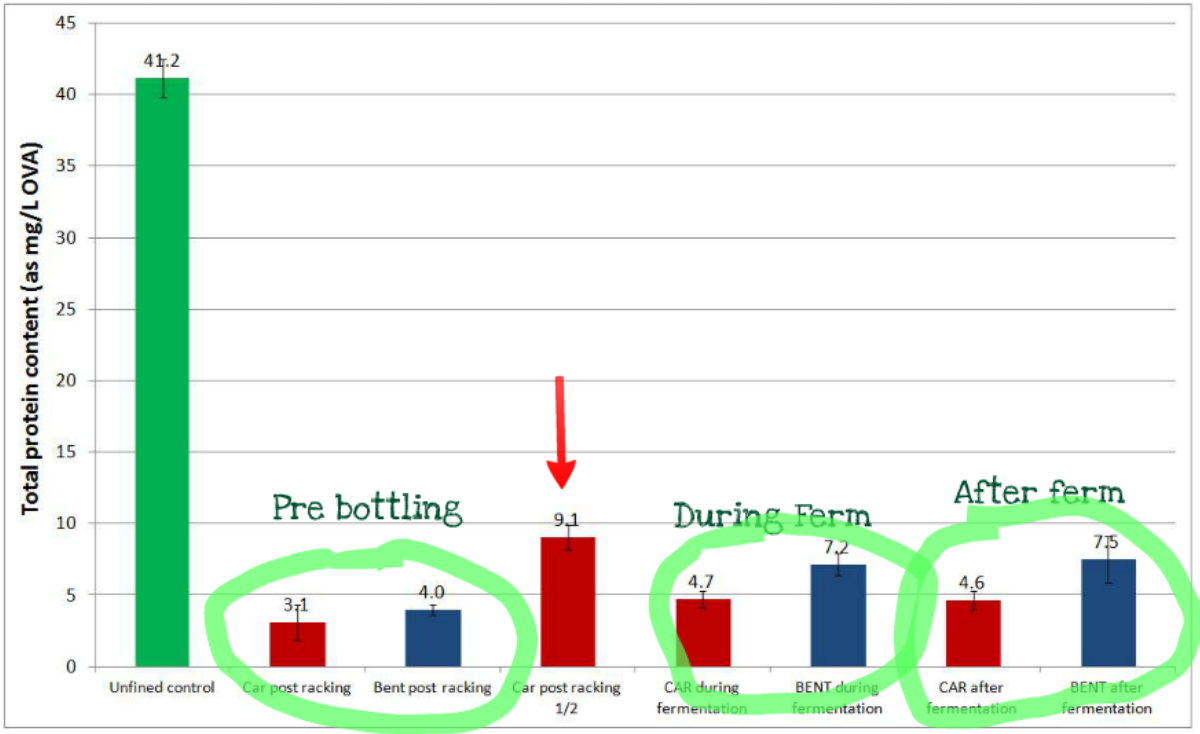
Proven to be effective in removing grape and wine proteins with low sensory and chemical impacts on treated wines

Protein content of bottled wines



Recent Findings

- If added at correct rate carrageenan can fully stabilise wines
- Need less carrageenan (1/2 dosage) if used in wines
- Possible filtration issues
- Optimum moment of application before or during ferment
- It produces less lees than bentonite (lower addition rate)
- Physicochemical parameters unchanged from controls

Marangon et al., 2012, AJGWR, 18, 194-202.
Marangon et al., 2013, JAFC, 61, 6516-6524.

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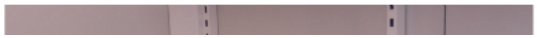
Zirconia

It is a metal oxide that can remove wine proteins

Pellet form used in batch additions experiments

Addition to juice better than to wine because stirring is required



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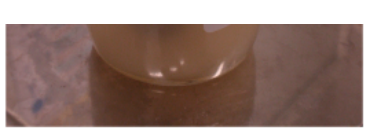

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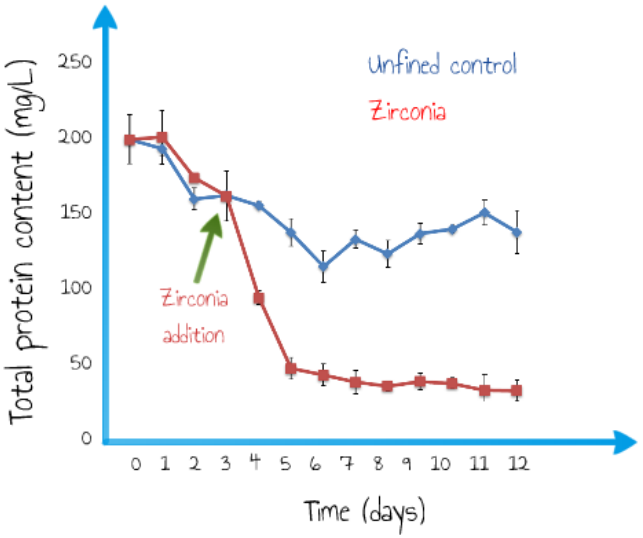

Lucchetta et al. 2013, AJEV, in press

Efficient in removing proteins
No main modification to the wine
No lees production, recyclable
Easily regenerated by washing

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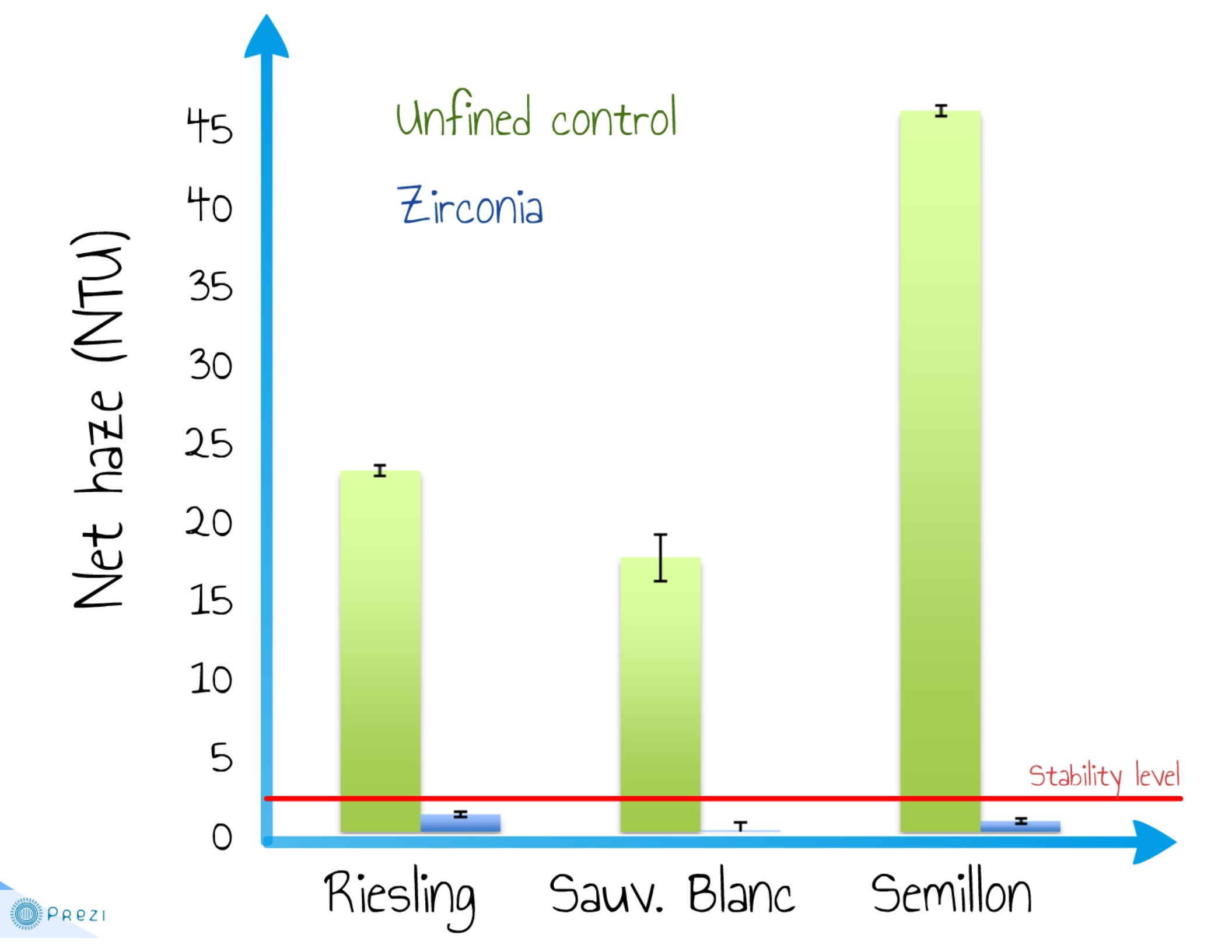
Net haze (NTU)
45
40
35
30
25
20
15
10
5
0
Unfined control
Zirconia
Stability level
Riesling
Sauv. Blanc
Semillon

Efficient in removing proteins

No main modification to the wine

No lees production, recyclable

Easily regenerated by washing

but...

high dosages required

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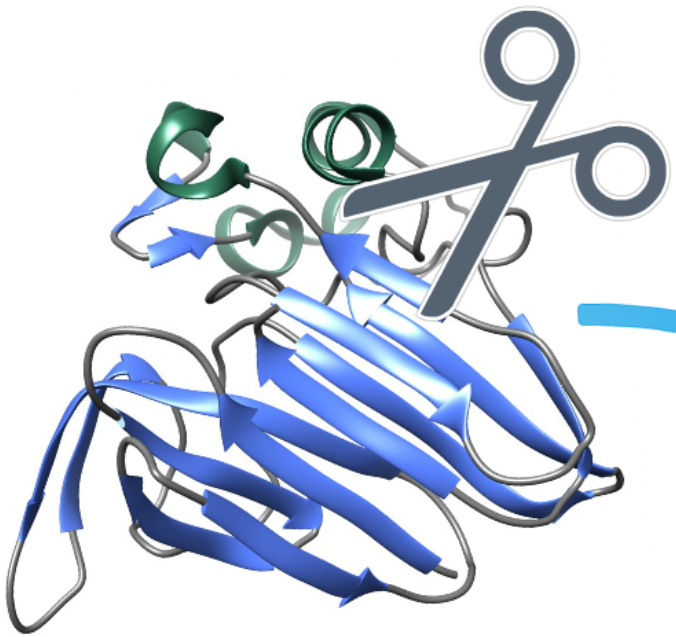
Proteins are globular



TLP - 4JRU

Resistant to proteases
in native status

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Unfolded by heat

Unfolding temperature of 55-62°C

Heat

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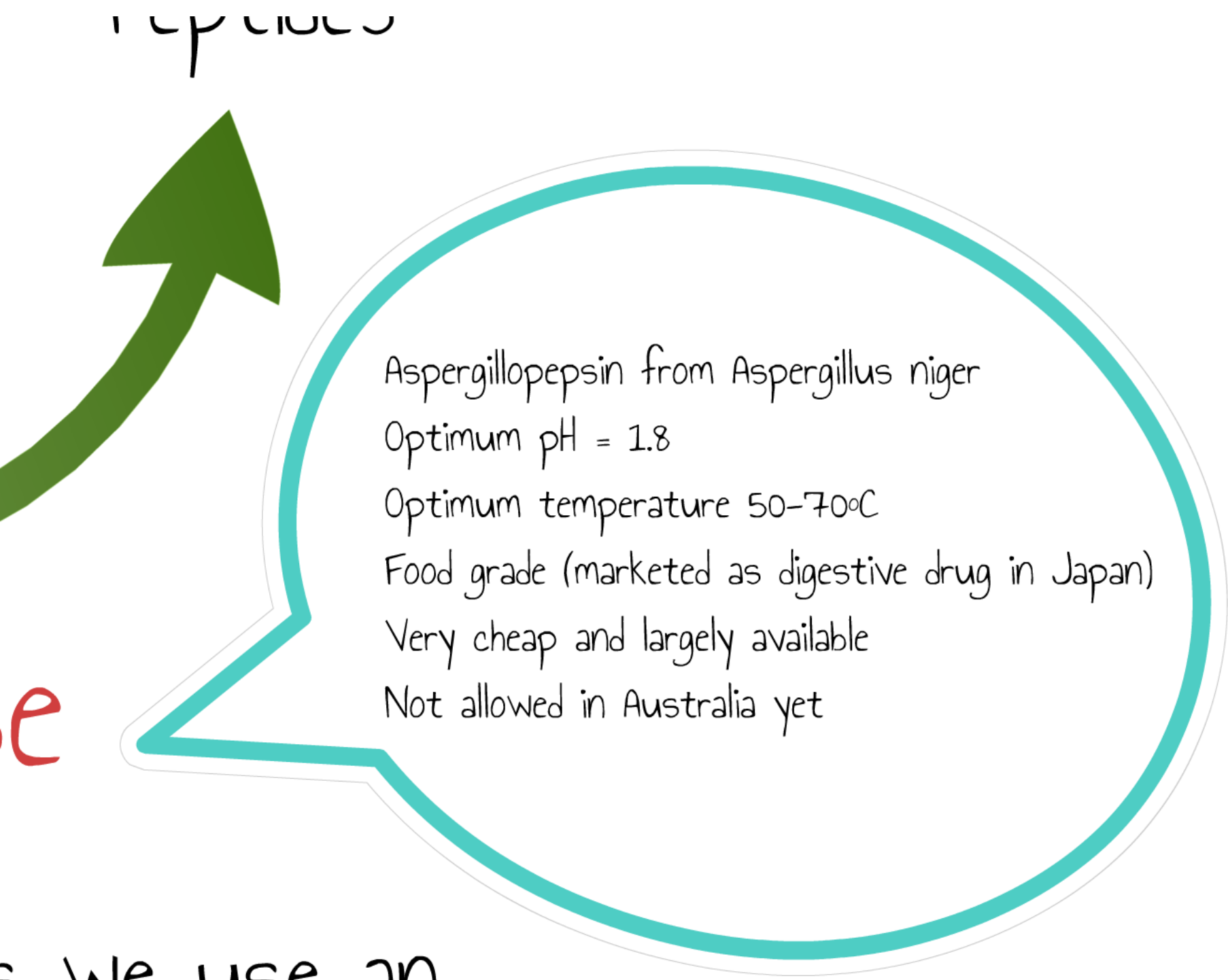

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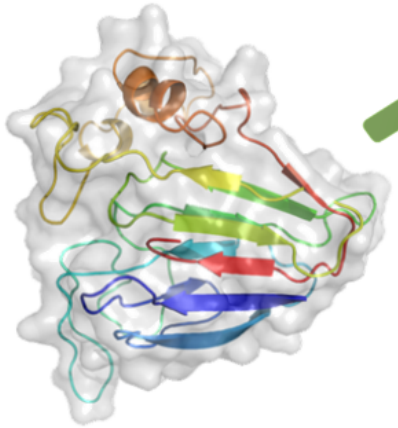
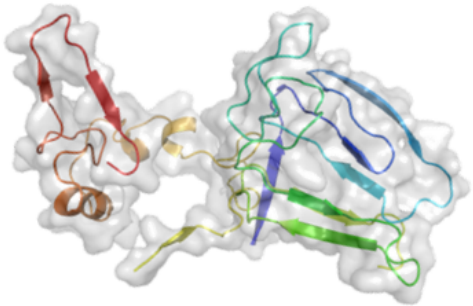


Native protein

Heat

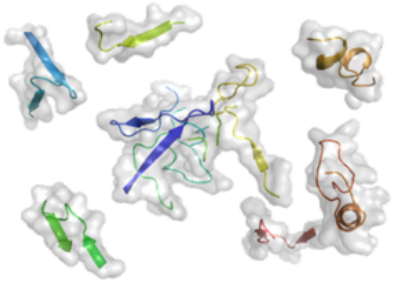
1 min @ 70oC



Unfolded protein

15 mg/L

Proctase



Peptides

Aspergillopepsin from Asperg
Optimum pH = 1.8
Optimum temperature 50-7
Food grade (marketed as dige
Very cheap and largely availa
Not allowed in Australia yet

Rather than finding an enzyme that works at winemaking conditions we use an enzyme at its optimum condition and with an easy substrate as an unfolded protein

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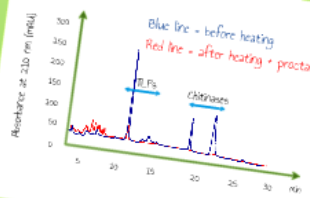
Proctase treatment

Done on clarified juice

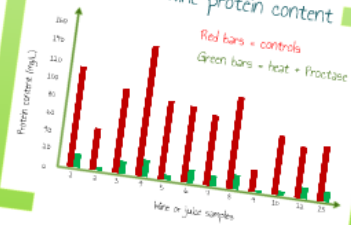
15 mg/L Proctase

Flash pasteurisation
(~1 minute, ~70°C)

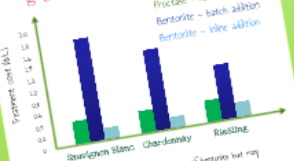
Effect on juice protein profile



Effect on wine protein content



Is this approach cost competitive?

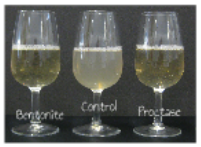


Less cost effective than in line dosage of bentonite but may be cheaper for small wineries.

What about the regulatory landscape?

- [illegible] are working to have Proctase approved for winemaking
- Enzymes of the same origin Pityrosyllus niger var. macrosporus are already approved as winemaking additives.
- Until NMN gets registration with FSANZ Proctase should not be used in commercial winemaking
- After registration it will be allowed in Australia likely from Vintage 2014 but wines won't be able to be exported to EU as work with GM to be done
- For more info contact [illegible]

Effect on heat stability



No chemical or sensorial effect compared to wine industry standards

How about the long term performances?

Wines stored at 15°C for 1 year:

- Proctase treated wines still haze free
- Unfined control was hazy
- Protein content mostly unchanged

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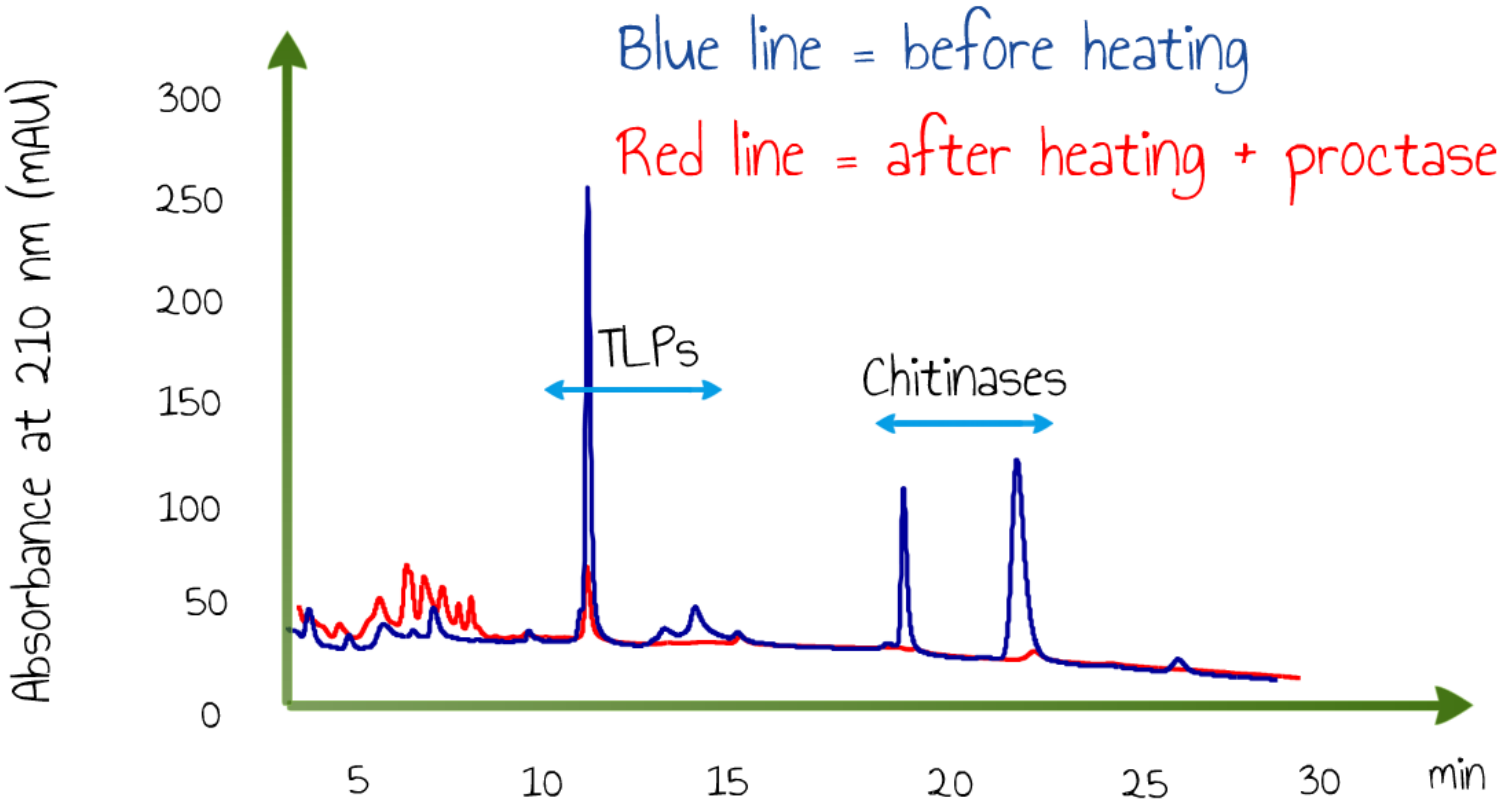

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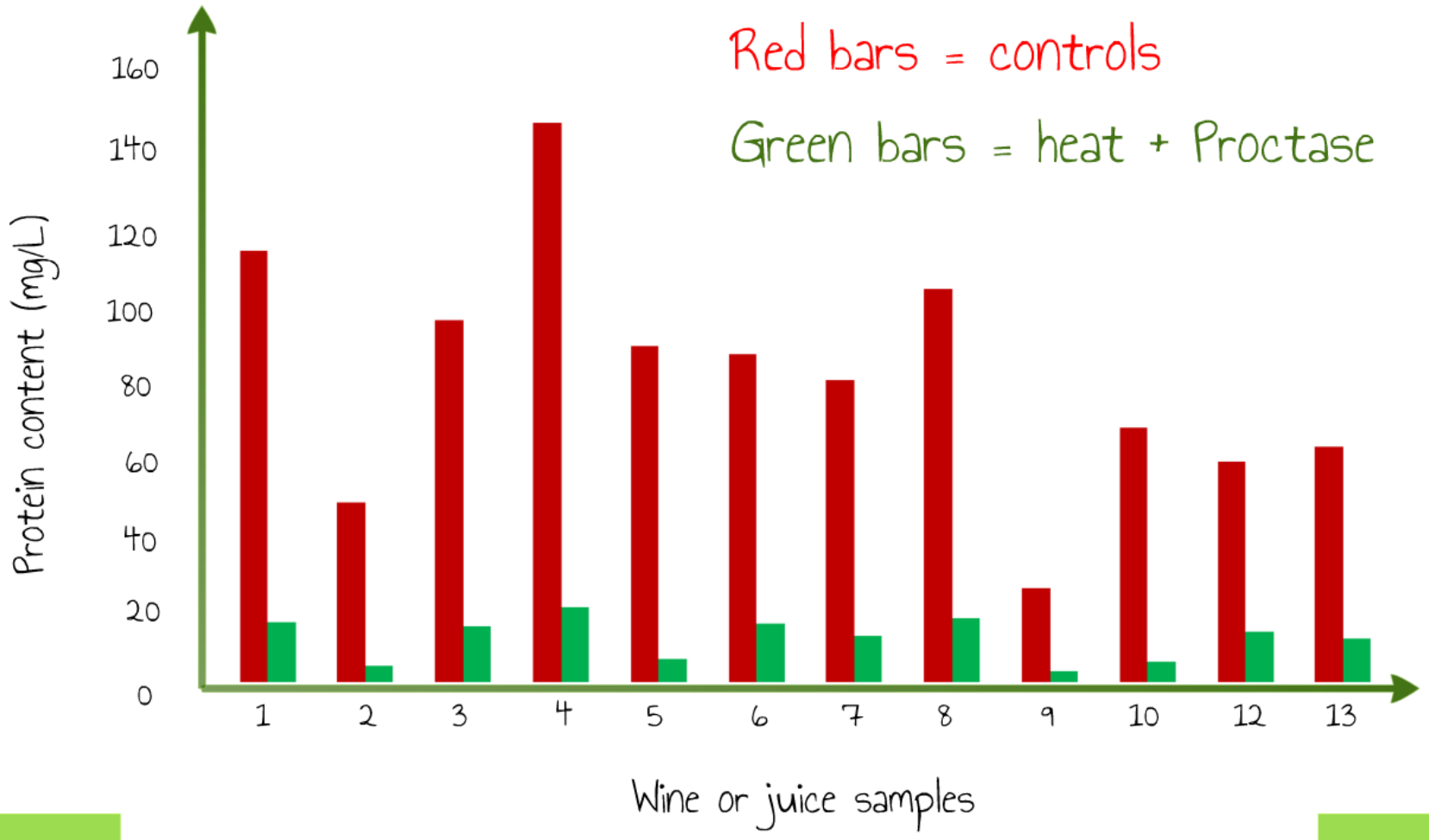
Effect on wine protein content



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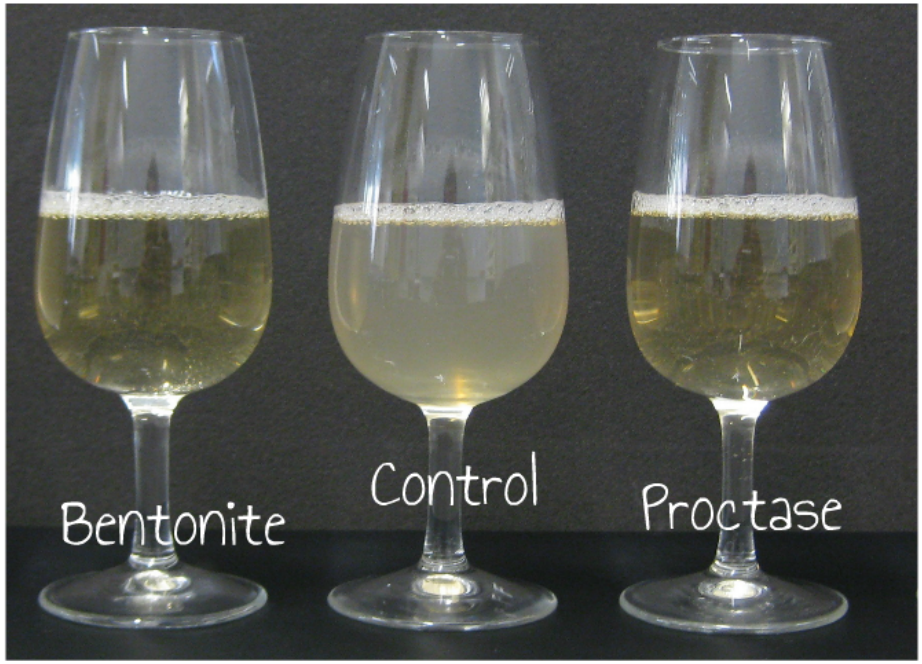

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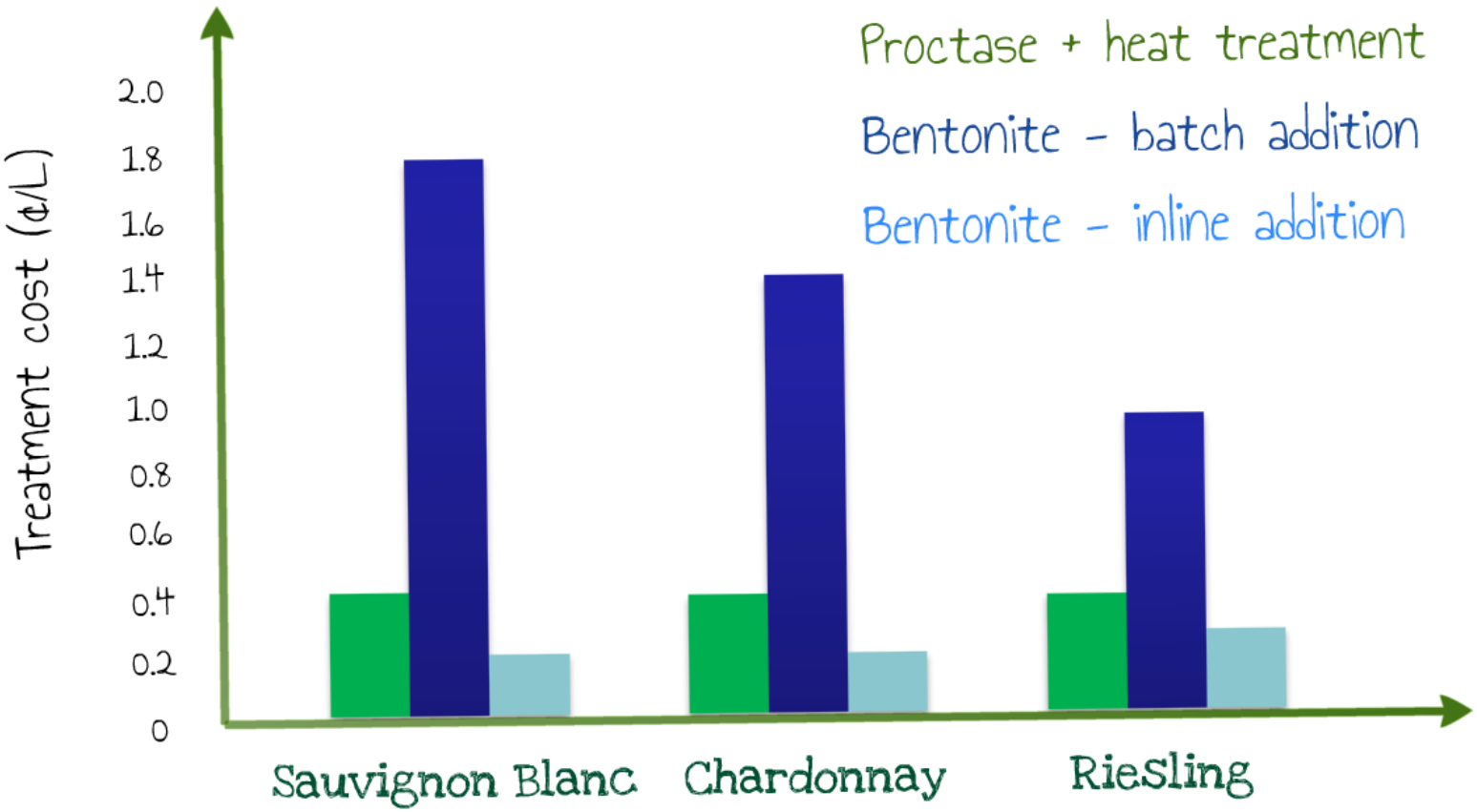

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- After registration it will be allowed in Australia, likely from Vintage 2014 but wines won't be able to be exported to EU so work with OIV to be done
- For more info contact ella.robinson@awri.com.au

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In general

Today there are still no viable alternative to bentonite because:

- Less efficiency than bentonite in removing proteins
- Often too expensive
- Fining times often too long
- Difficulties in passing from lab scale to winery scale
- Possible negative effects on the sensory profile of wines

but...

- Carrageenan and Zirconia are promising
- flash pasteurisation + proctase should become viable in the near future

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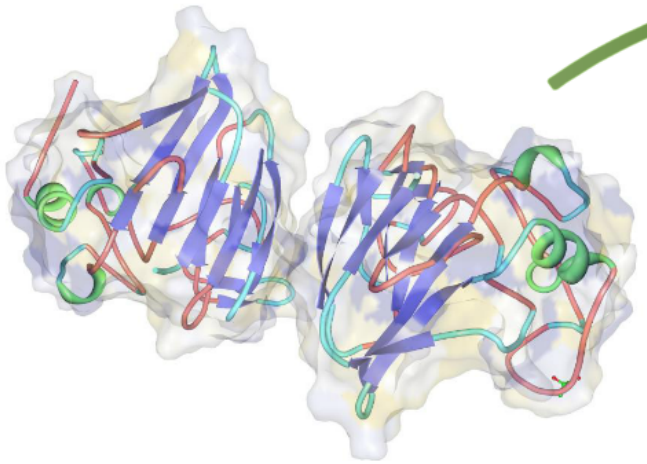
Other approaches?



VVTL1 - 4h8t

Find some enzymes:

- cold active enzyme
- proteases from yeast?
- database search against solved structure

Unfold proteins in other ways

- Microwave

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Unfold proteins in other ways

- Microwave
- High pressure treatments
- pulsed electric field

hents??

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Other adsorbents??

- Zirconia
- Carragenan
- Surface Engineered Silica
- Chitin
- Tannin additions
- Something else??

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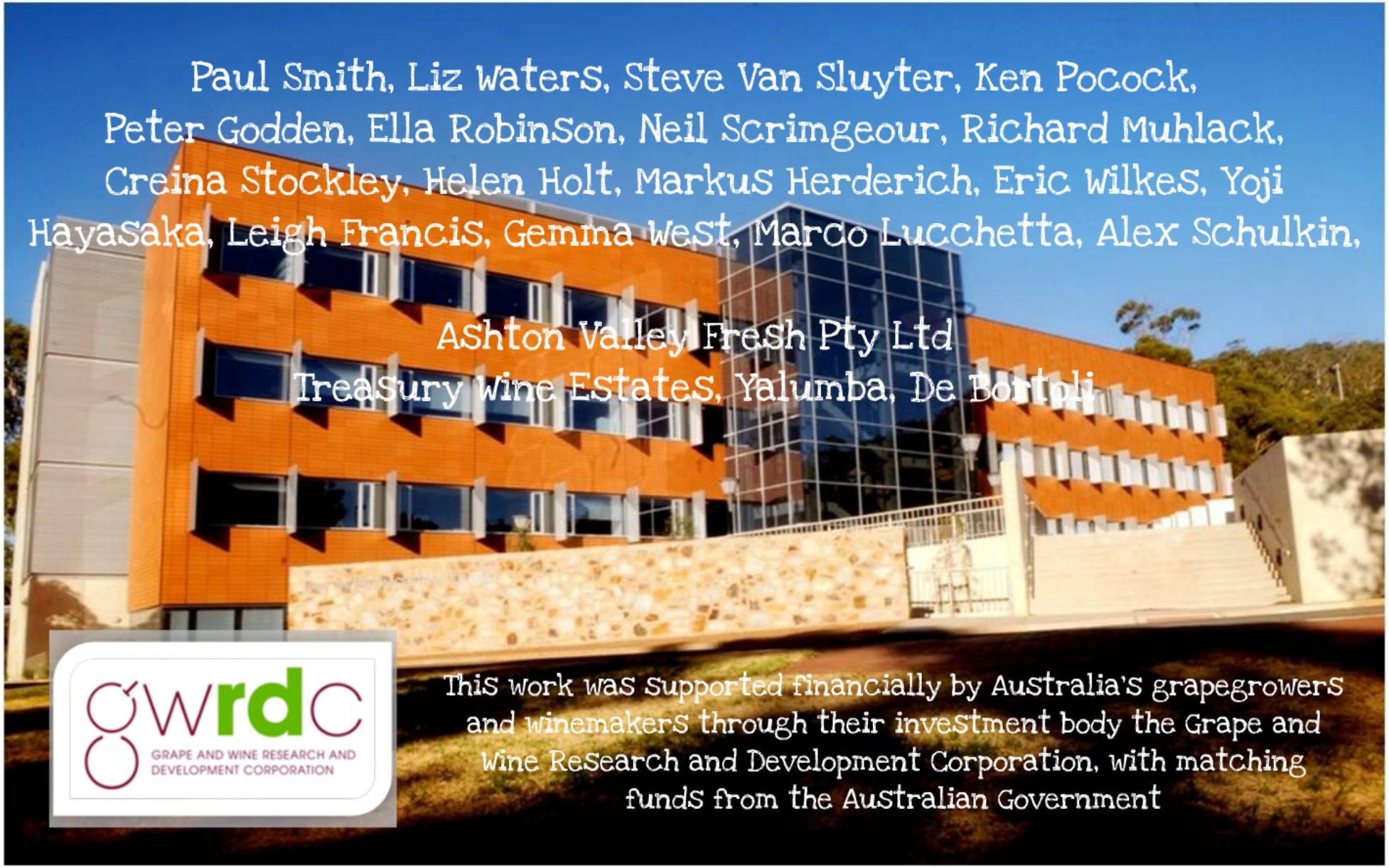
Acknowledgments



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