Managing the risk of protein haze formation in wines

Dr Matteo Marangon

15/04/2016

AWRI

Plumpton College

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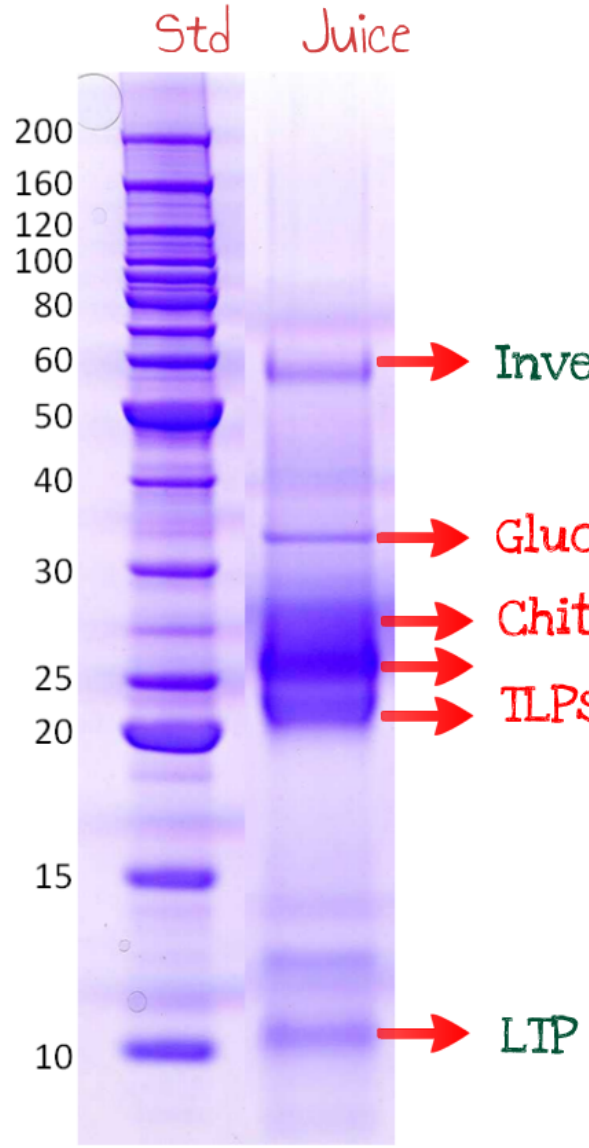
The grape proteins



Invertases

Glucanases

Chitinases

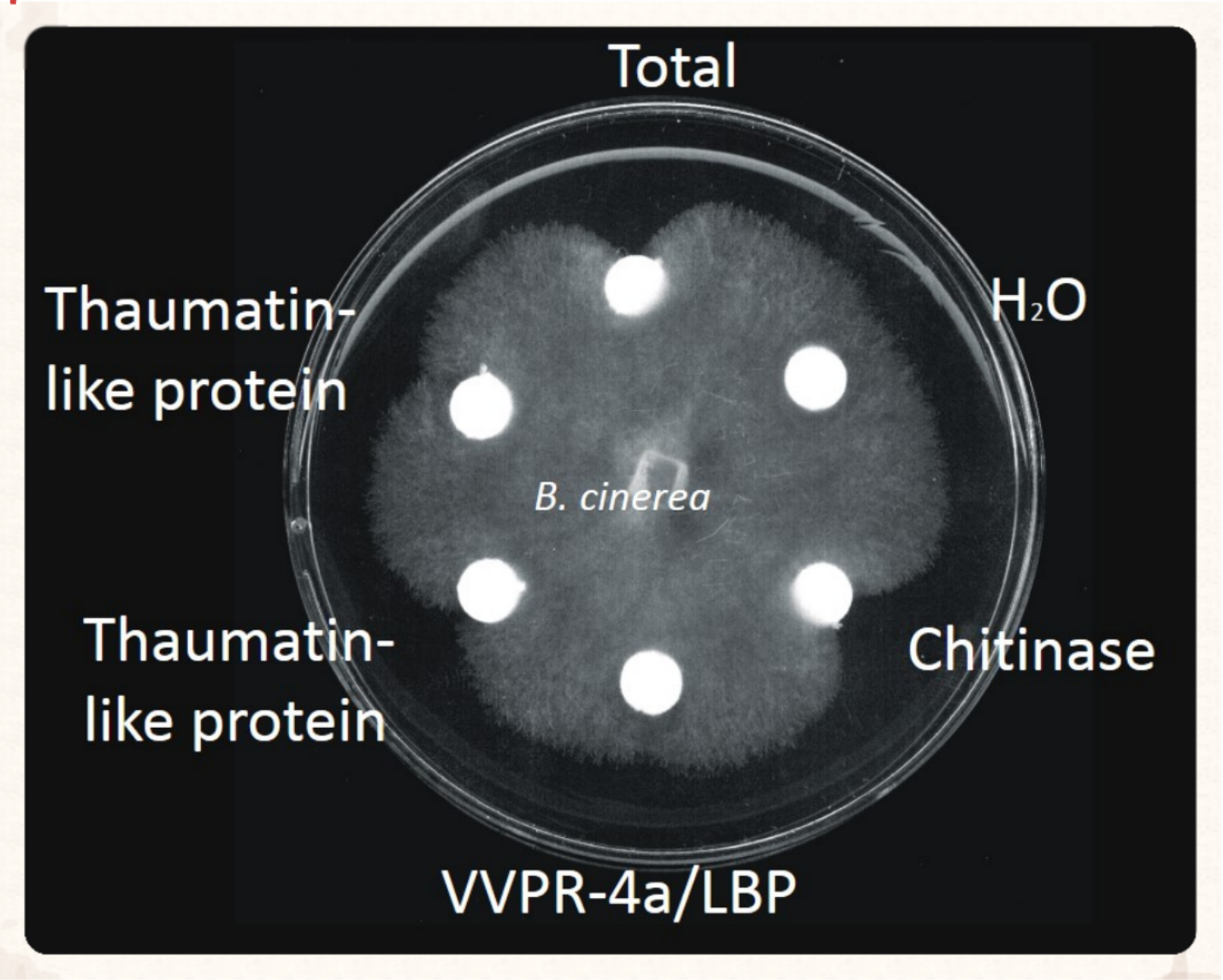
TLPs

LTP

Heat unstable!
found in hazes

- PR-proteins = antifungal activity
- Resistance to proteolysis
- Stable at low pH (wine = 3-3.5)
- Tendency to get insoluble during wine storage

PR-proteins characteristics

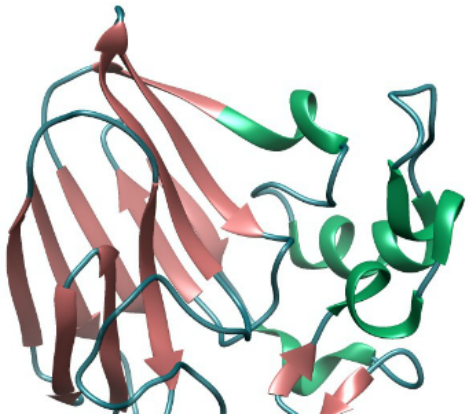


What do we know about PR-proteins?

- All grape cultivars synthesize PR-proteins during ripening
- The main PR-proteins are **Thaumatins** and **chitinases**
- PR-proteins are the main responsible for protein haze formationn white wines

PR-proteins characteristics:

- Very resistant to proteolysis
- High stability at low pH



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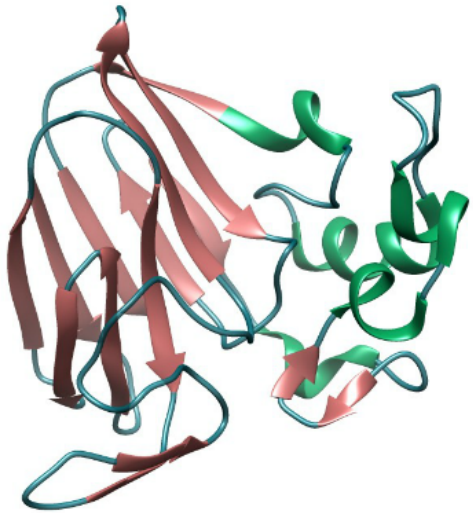
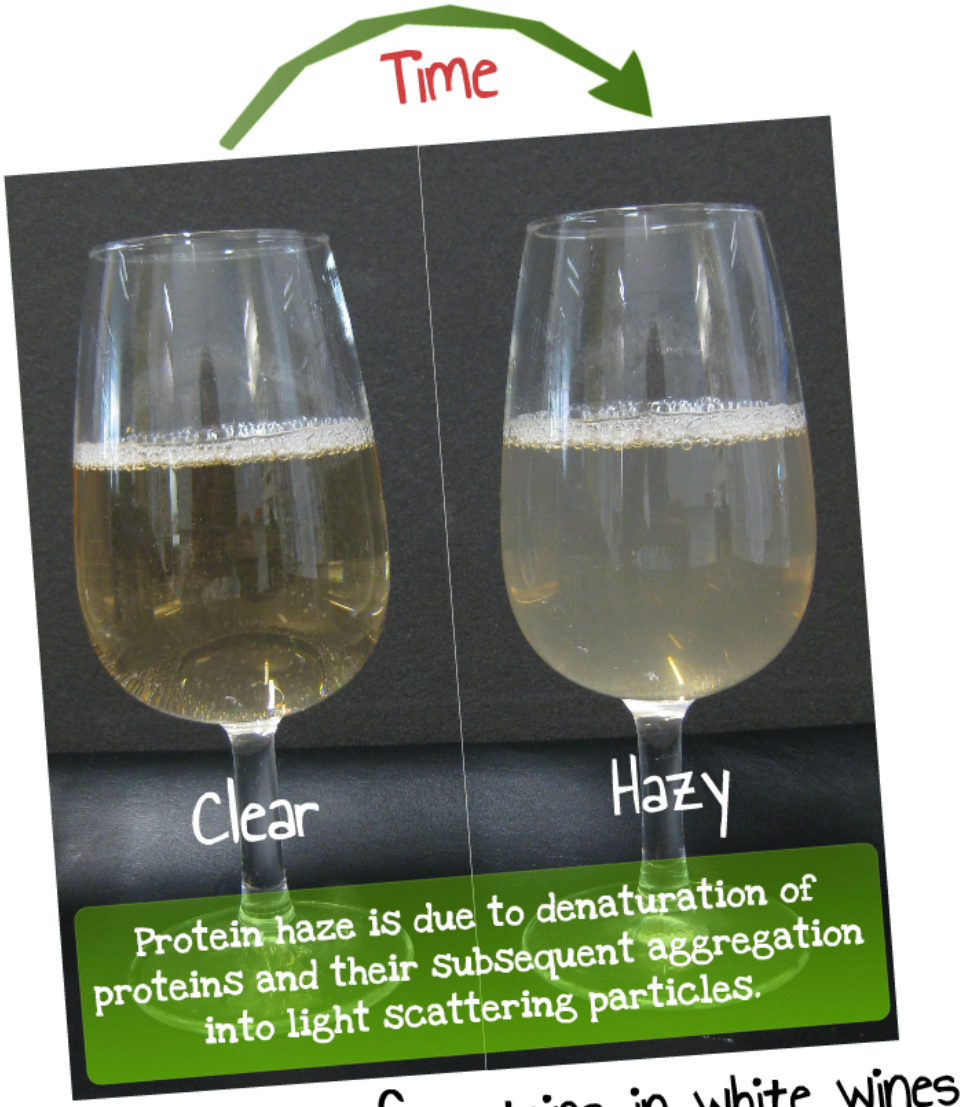


TLP 4L5H

↓↓

Vinification selectively purifies PR-proteins

Which wine would you drink?



50-400 mg/L of proteins in white wines

Factors affecting protein concentration in wines

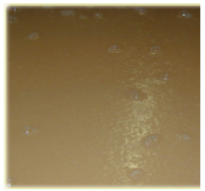
Grape maturity

Skin contact

Water stress

Fungal infections

Fermentation

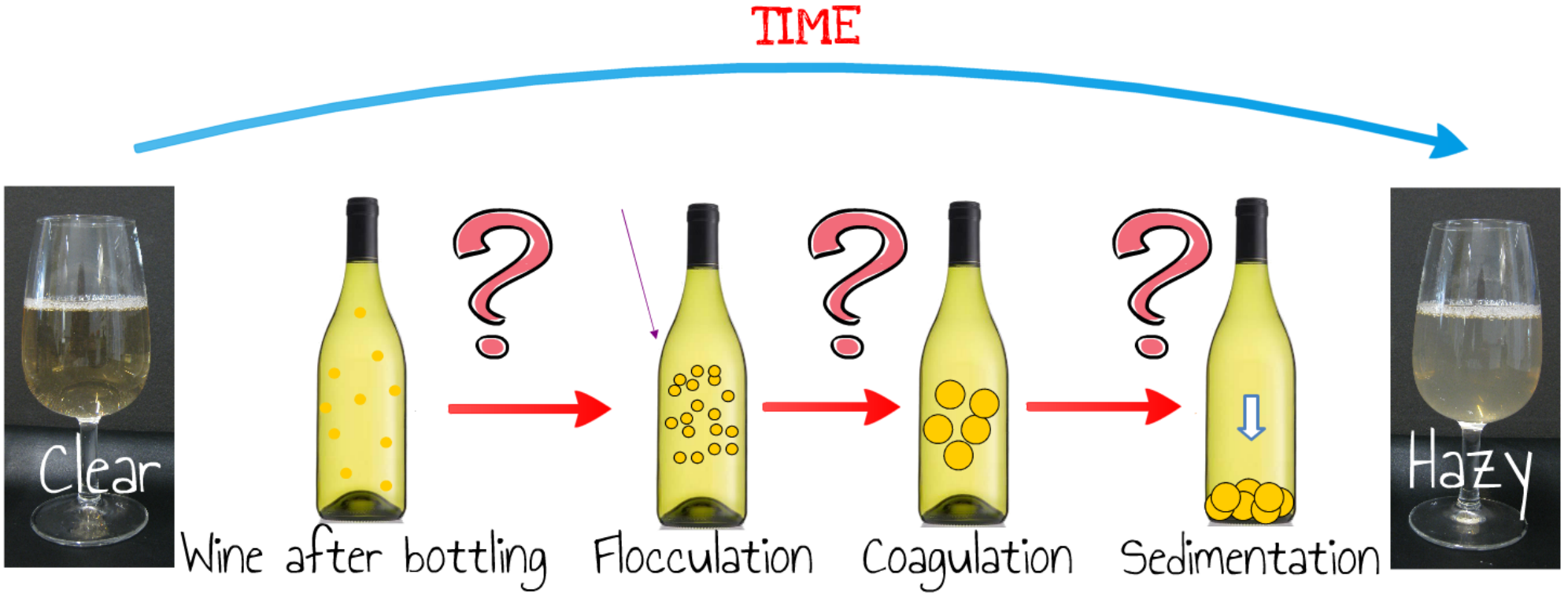


Bentonite

Why are wines getting turbid??

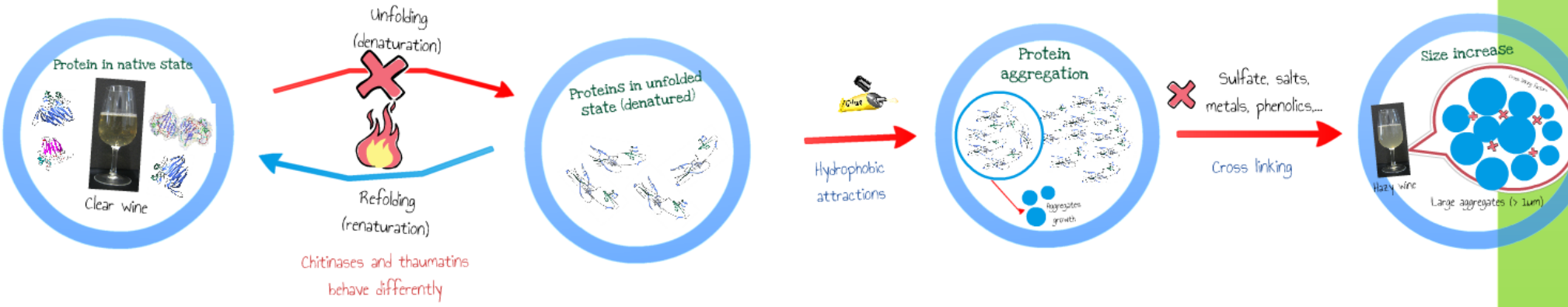
- Lack of removal of heat unstable proteins from musts and wines
- Wines stored at inappropriate temperatures (too hot, fluctuating temperatures)
- Errors in calculating Bentonite addition rates
- Modification of wine compositions (e.g. addition of MCR o fortification with alcohol)

'Life cycle' of proteins in wine

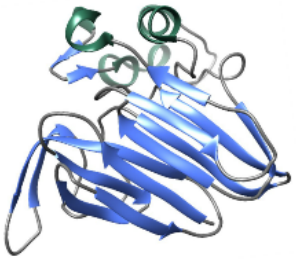


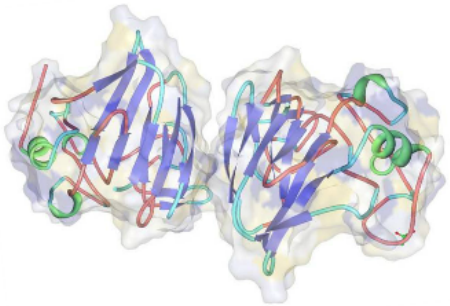
How can these question marks be solved??

Detailed mechanism of haze formation



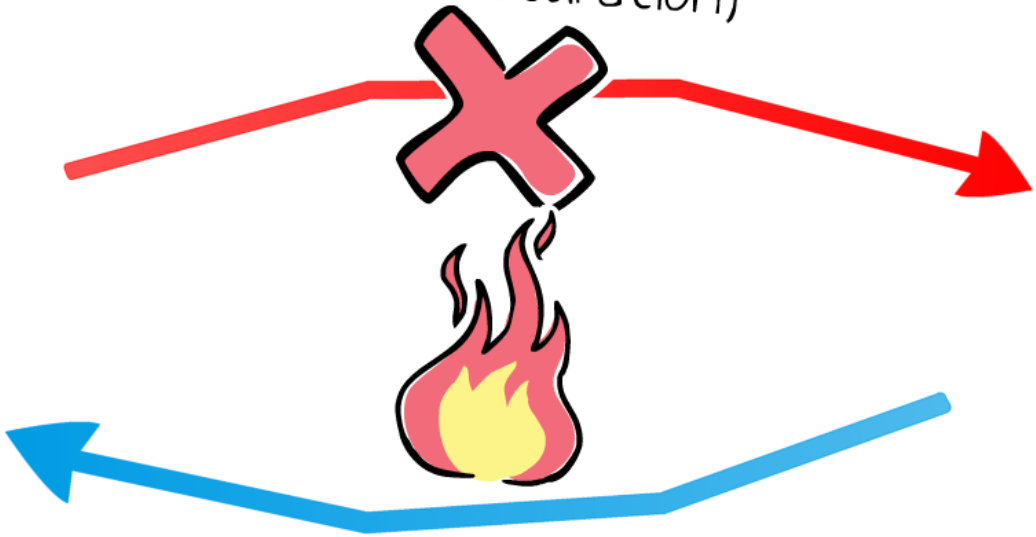
Protein in native state





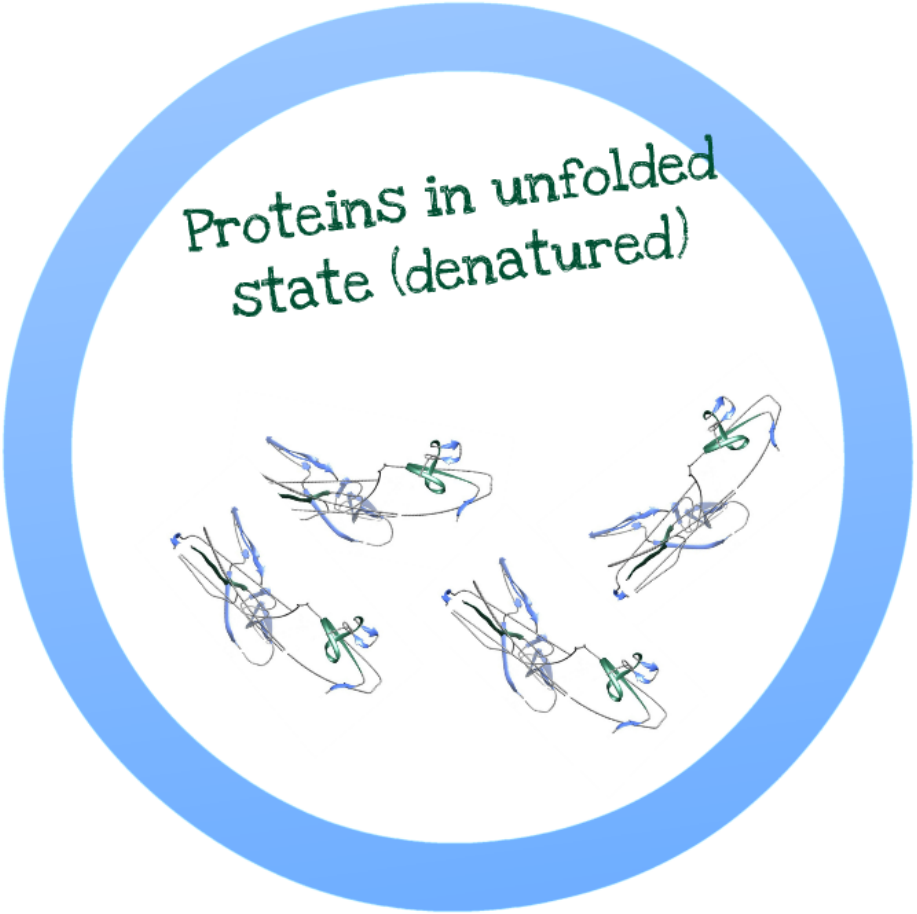
Clear wine

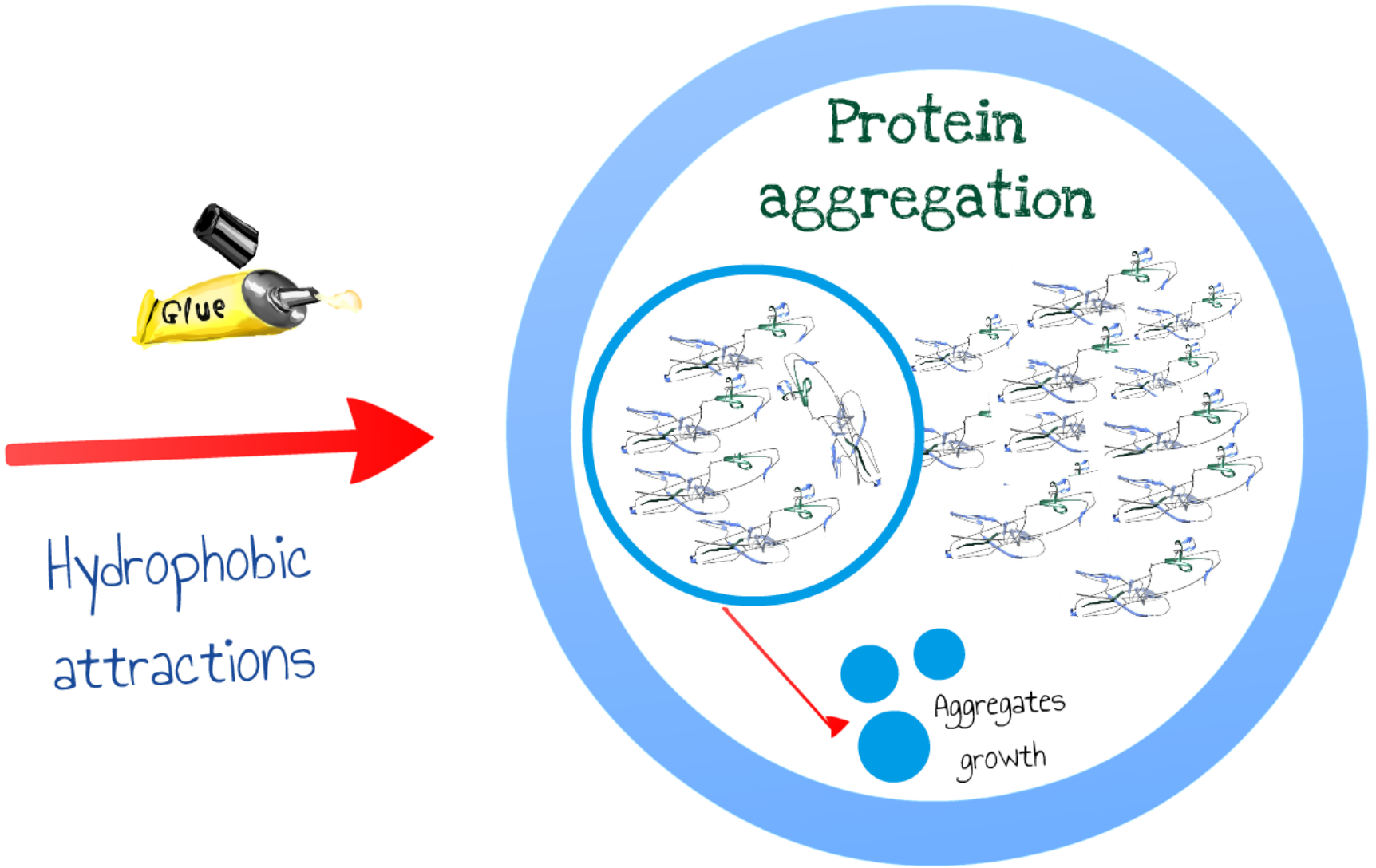
Unfolding
(denaturation)



Refolding
(renaturation)

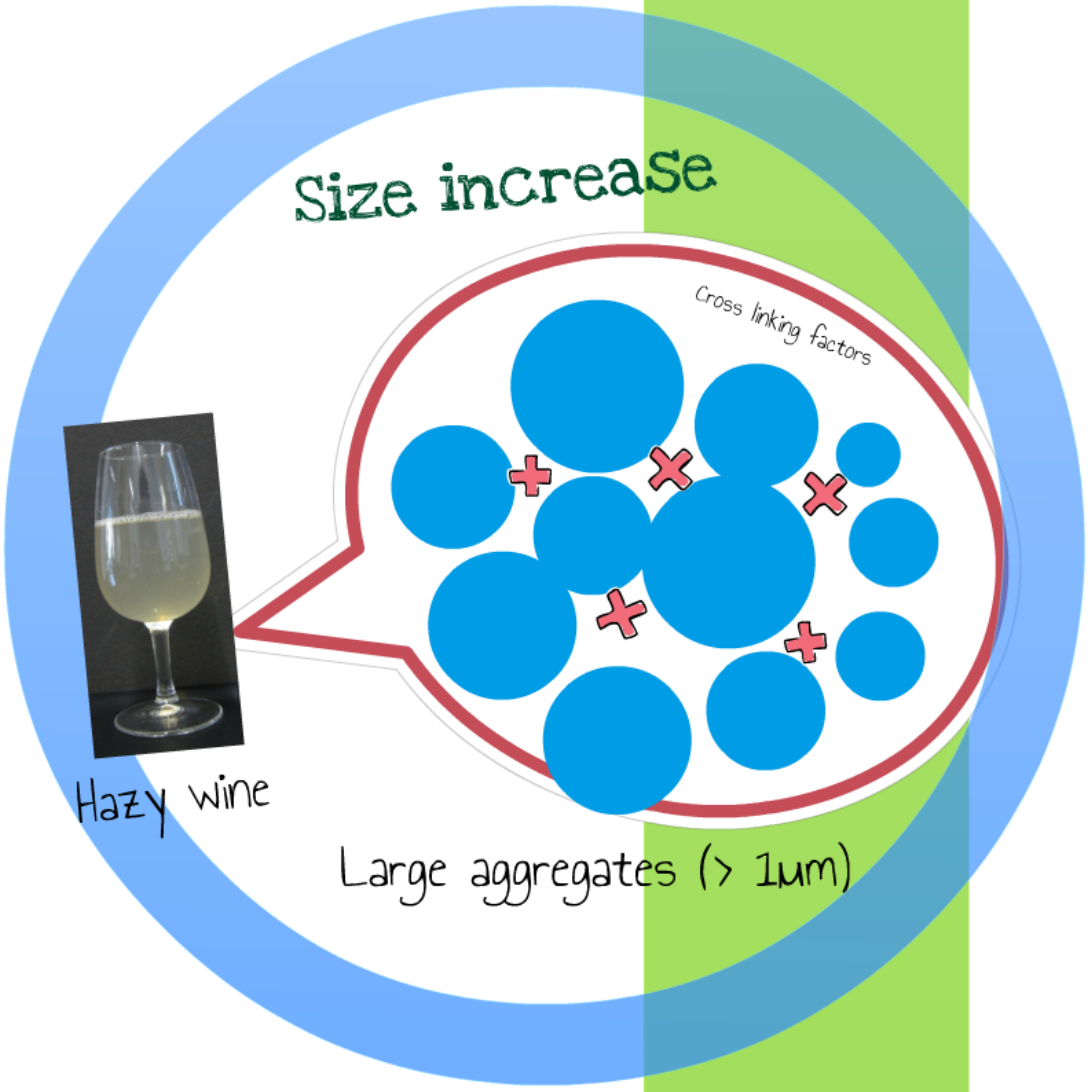
Chitinases and thaumatins
behave differently




Glue
Hydrophobic
attractions
Protein
aggregation
Aggregates
growth

Sulfate, salts,
metals, phenolics,...

Cross linking



Why is it important to have these info?

New knowledge useful for:

- Set up better tools to predict the potential of haze formation in wines
- Study targeted alternatives to bentonite to prevent haze formation in white wines

Positively Charged

TLP 4JRU

Current solution

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

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

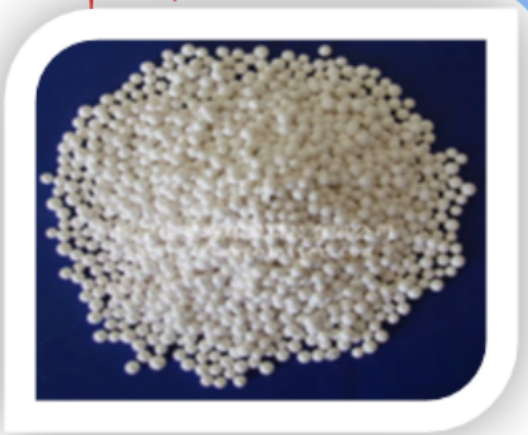
Cost for the industry:
1 Billion dollar/year worldwide!!

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

Bentonite alternatives proposed

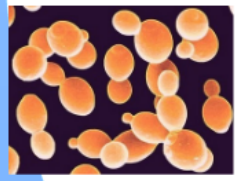
Adsorption of chitinases on chitin substrate

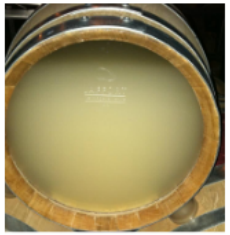
(Vincenzi et al., 2005)



Adsorbent resins (Dowex, Amberlite, ...)

(Gump and Huang, 1999)



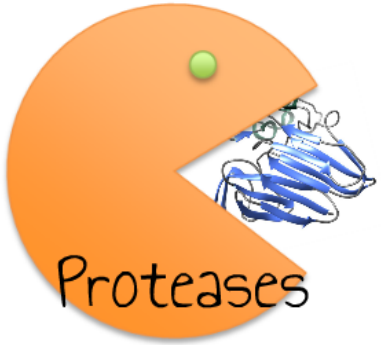


Mannoproteins, Haze protective factor

(Schmidt et al, 2010)

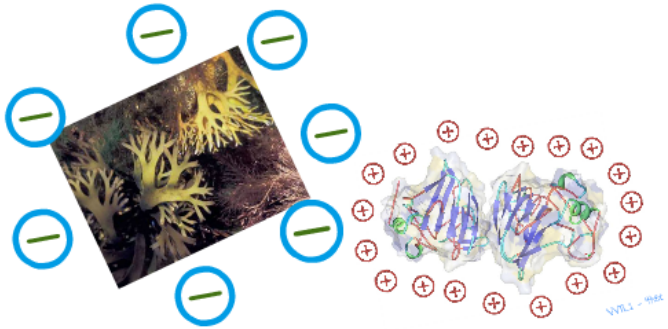
Zirconia dioxide

(Pashova et al., 2004)



Carrageenan

Carragenan is a hydrocolloid of the red seaweeds cell walls
Used for protein stabilisation and copper removal in beer

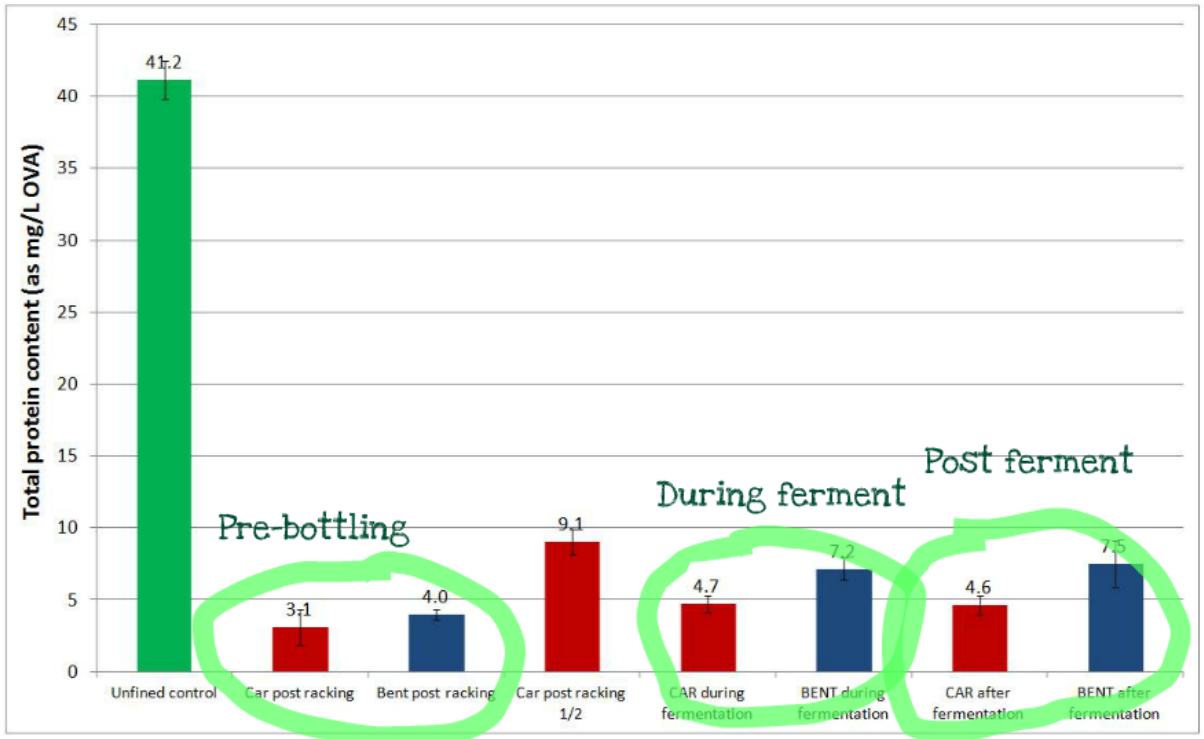


Seaweeds farming

Effective in removing grape and wine proteins with low sensoral and chemical impact ion treated winesi

Effective in removing grape and wine proteins with low sensoral and chemical impact ion treated winesi

Protein content of bottled wines



Recent discoveries

- When added at the right dosage carrageenan **can fully stabilise wines**
- Lower carrageenan addition rate needed (1/2 dosage) when used in wines
- Can cause **filtration problems** (work in progress)
- Best if added in pre-fermentation
- **Less lees** produced than bentonite
- Physico-chemical and sensorial **parameters unchanged** from control

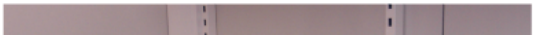
Marangon et al., 2012, AJGWR, 18, 194-202.
Marangon et al., 2013, JAFC, 61, 6516-6524.

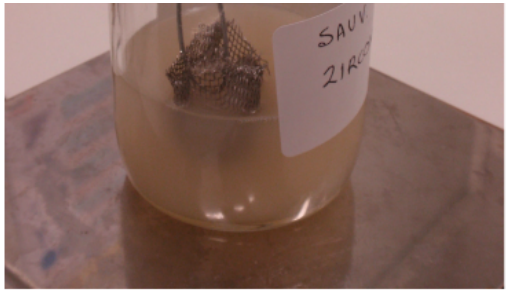
Zirconia

Metal oxide that binds wine proteins

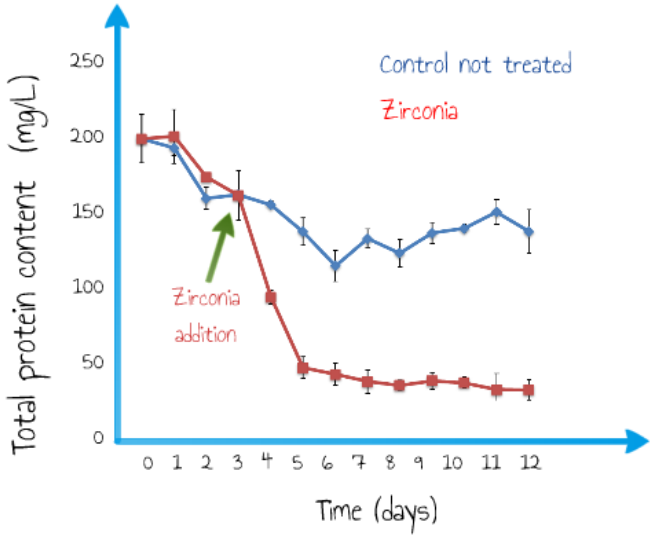
Used in pellet form

Best when added to fermenting musts as there is natural mixing

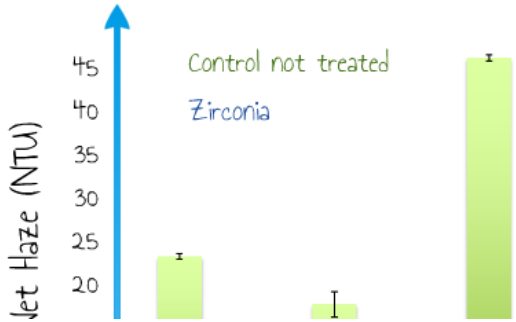




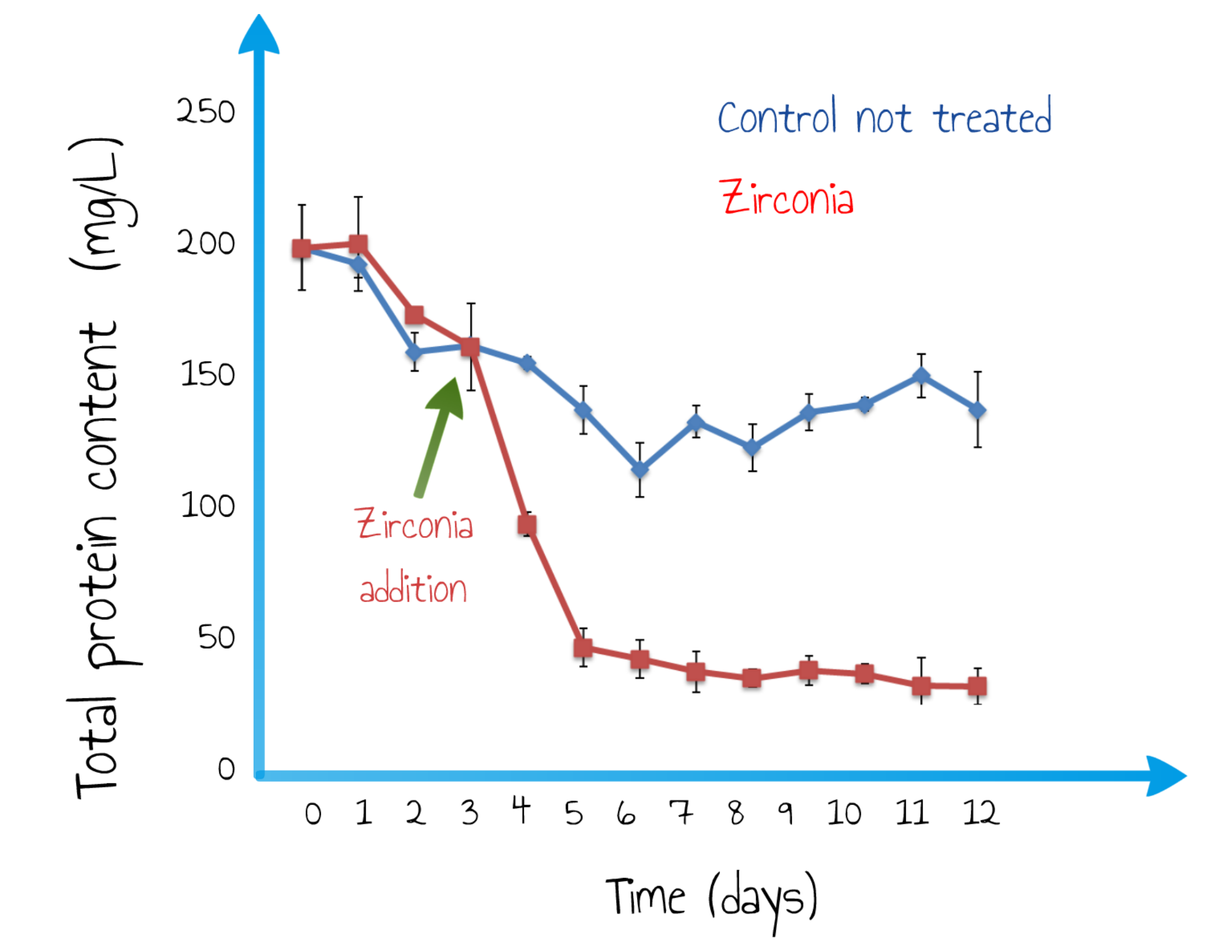
Best when added to fermenting musts as there is natural mixing

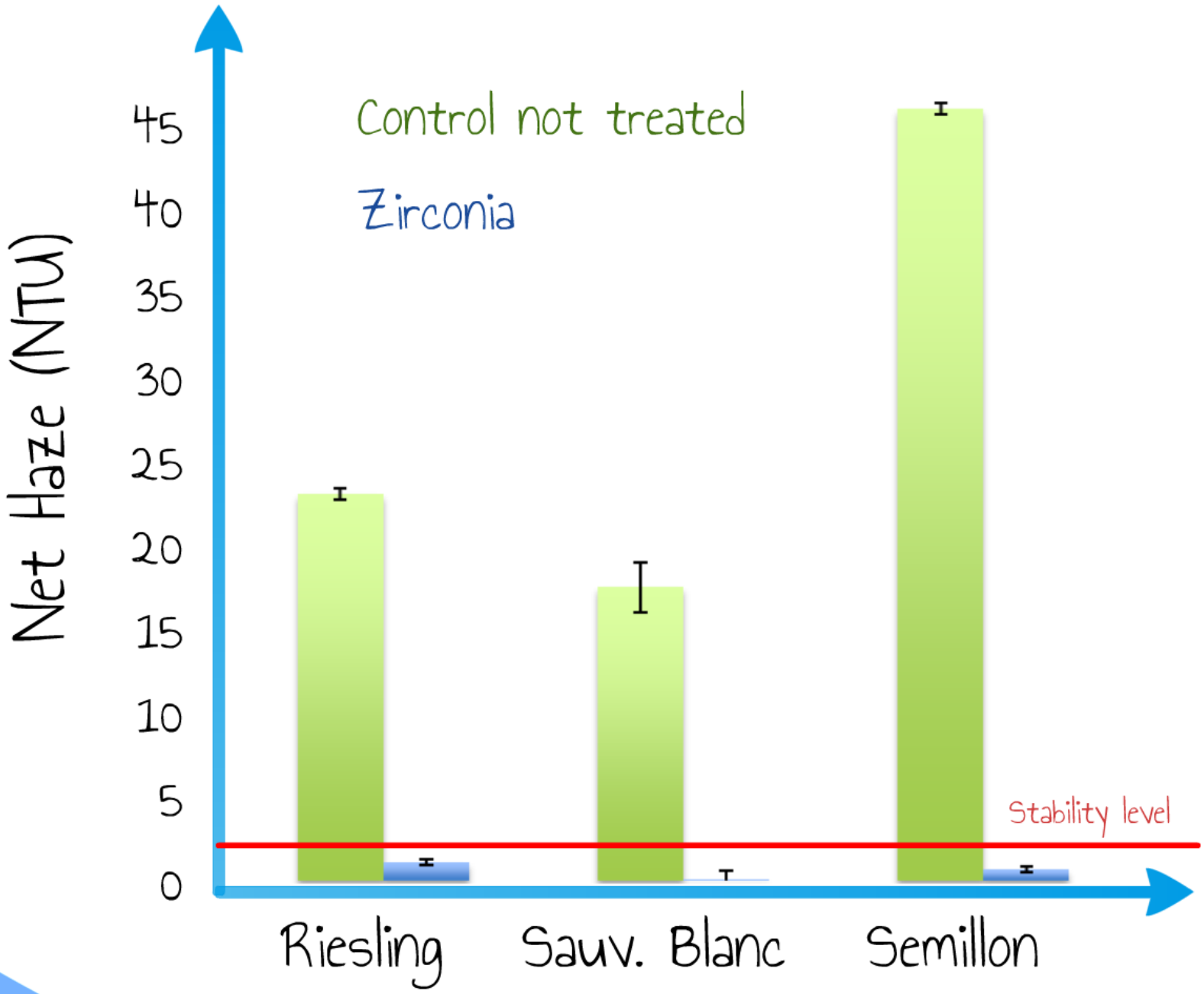


Lucchetta et al. 2013, AJEV



Efficient in removing proteins
No significant modification of wines
No lees produced
Reusable after washing


Total protein content (mg/L)
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (days)
Control not treated
Zirconia
Zirconia addition


Net Haze (NTU)
45
40
35
30
25
20
15
10
5
0
Control not treated
Zirconia
Stability level
Riesling
Sauv. Blanc
Semillon

Efficient in removing proteins

No significant modification of wines

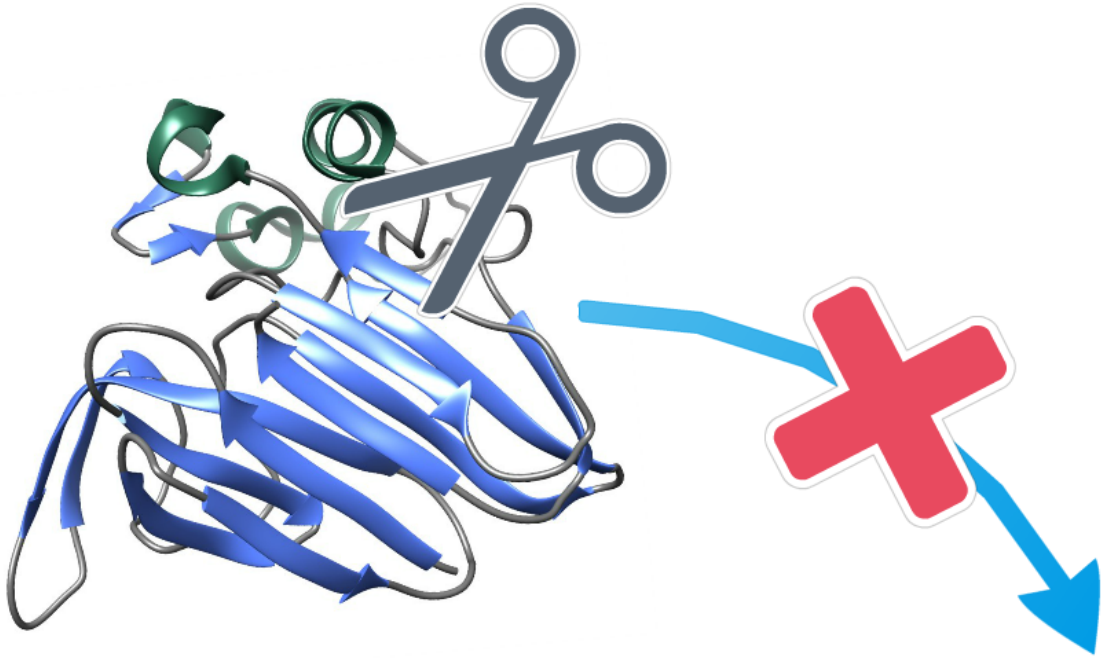
No lees produced

Reusable after washing

BUT...

High rates of addition required

Proteins are globular

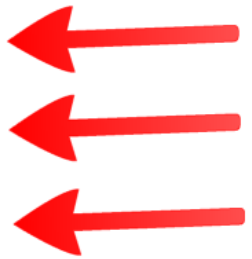


TLP - 4JRU

Resistant to proteases in native state

Unfolded by heat

Protein	Melt temperature
Chitinases	55℃
TLP	56-62℃
β-glucanases	<40℃
Invertases	81℃
LTP	>80℃



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

Protein	Melt temperature
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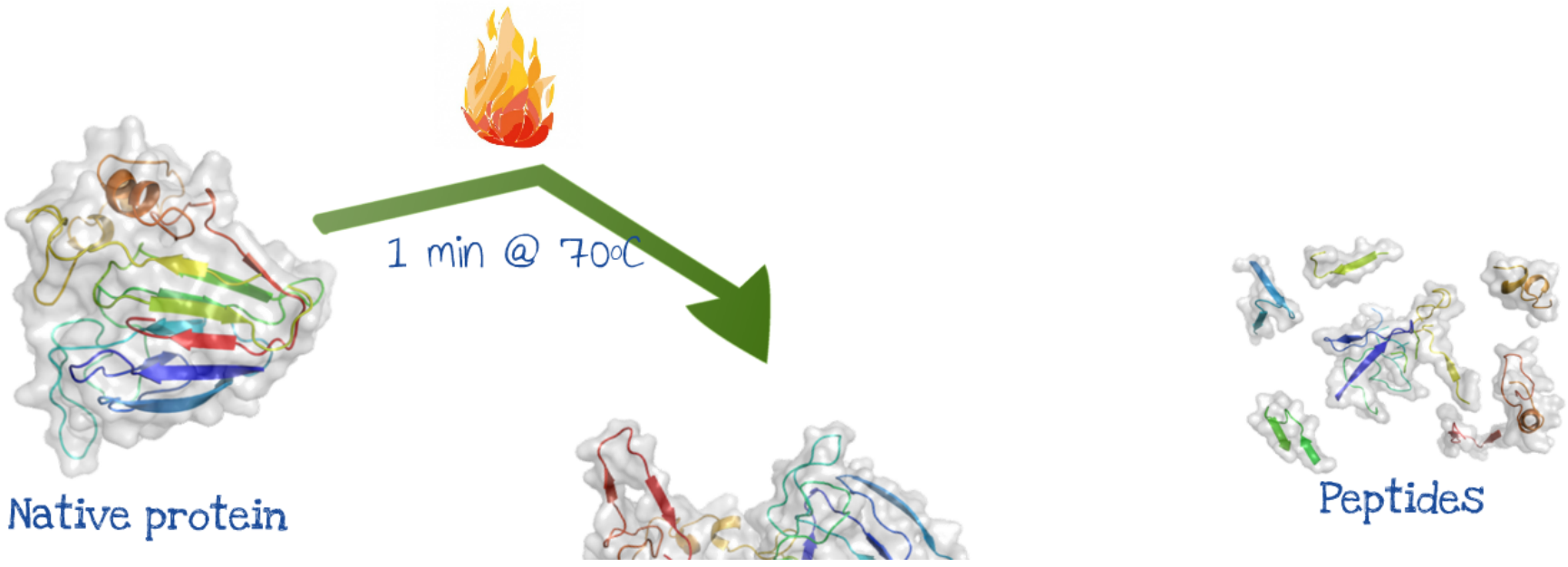
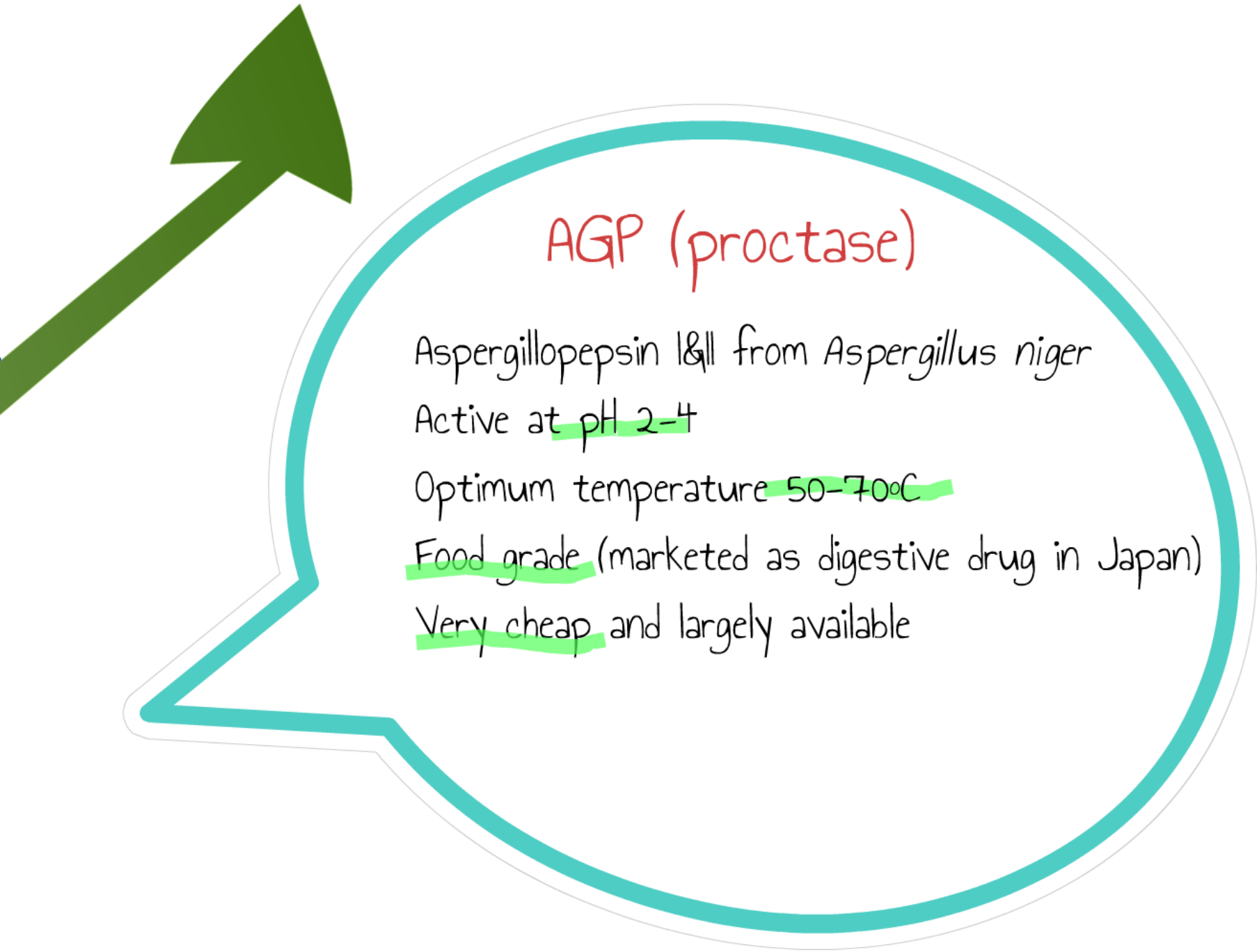
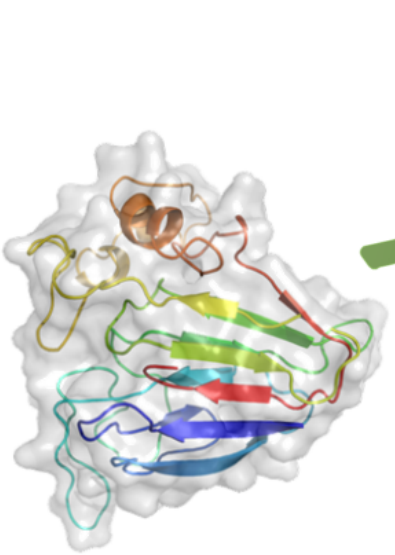


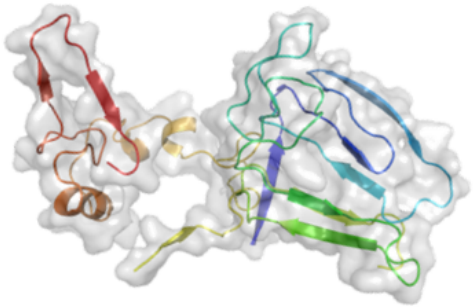

AGP (proctase)
Aspergillopepsin I&II from Aspergillus niger
Active at pH 2-4
Optimum temperature 50-70°C
Food grade (marketed as digestive drug in Japan)
Very cheap and largely available

enzyme at its optimum condition and with an easy substrate as an unfolded protein



Native protein

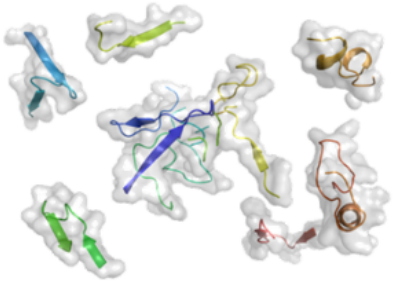
1 min @ 70°C



Unfolded protein

15 mg/L

AGP



Peptides

AGP (proctase)

Aspergillopepsin I&II from Asper

Active at pH 2-4

Optimum temperature 50-70°C

Food grade (marketed as digesti

Very cheap and largely available

Treatment on clarified juice

Preliminary lab- experiments to define conditions:

15 mg/L AGP

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

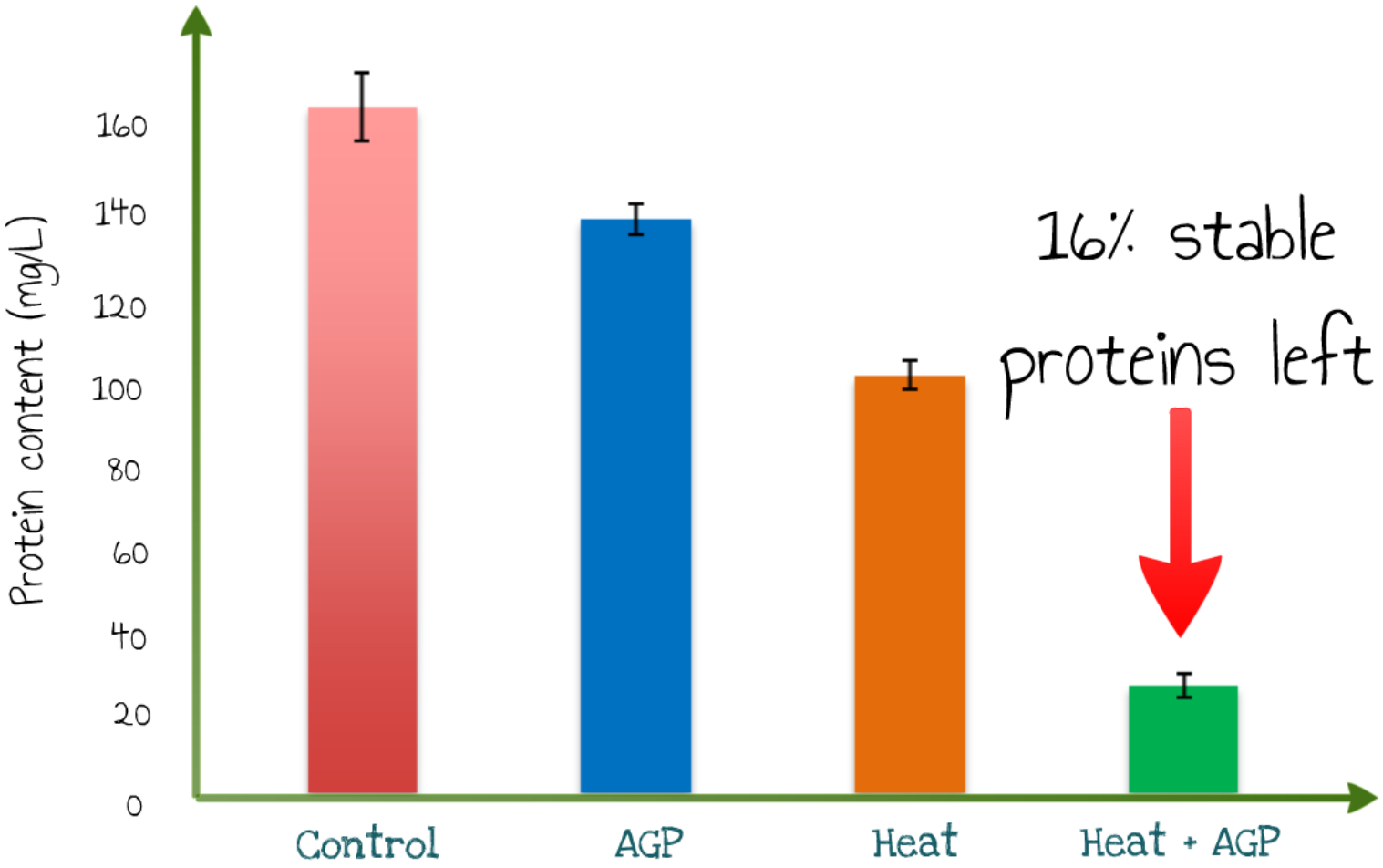
Two 1000L unfined juices

80 L fermentations in triplicate

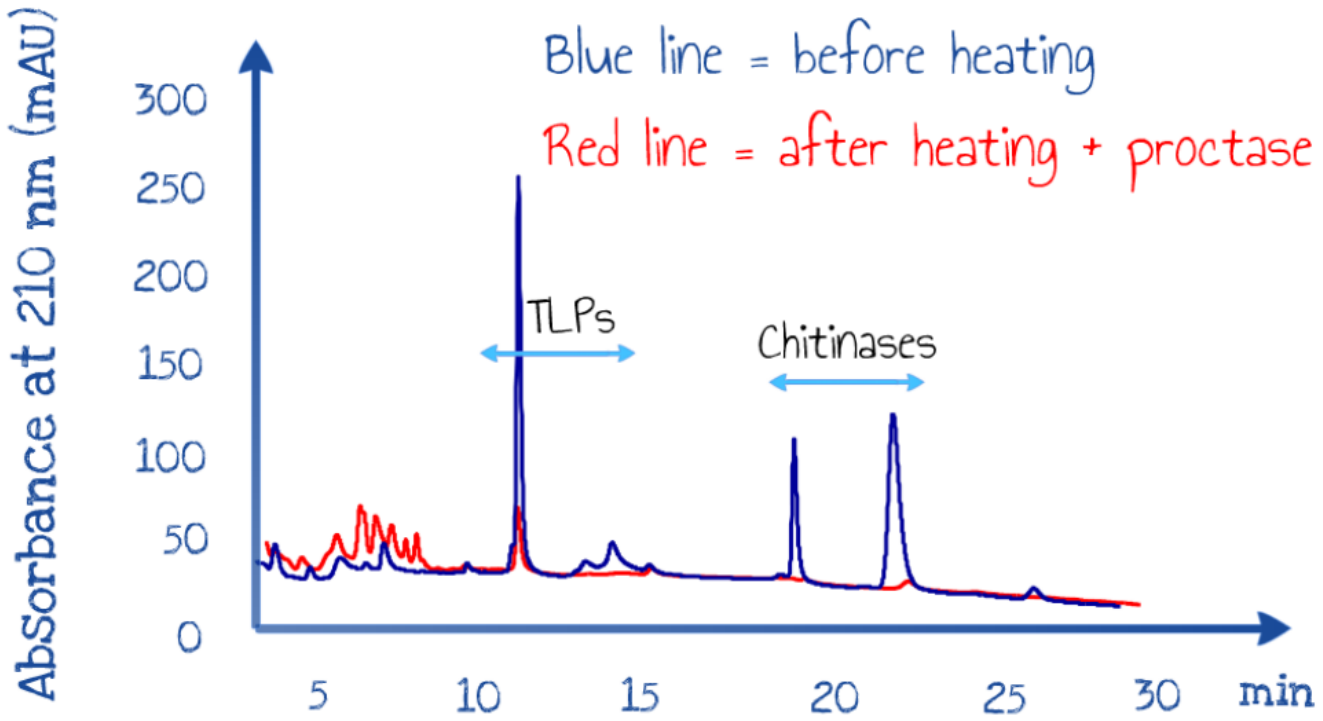
Five treatments:

1. Unheated control
2. Unheated control + Bentonite
3. Unheated + AGP
4. Heated
5. Heated + AGP

Treatment effect on protein content

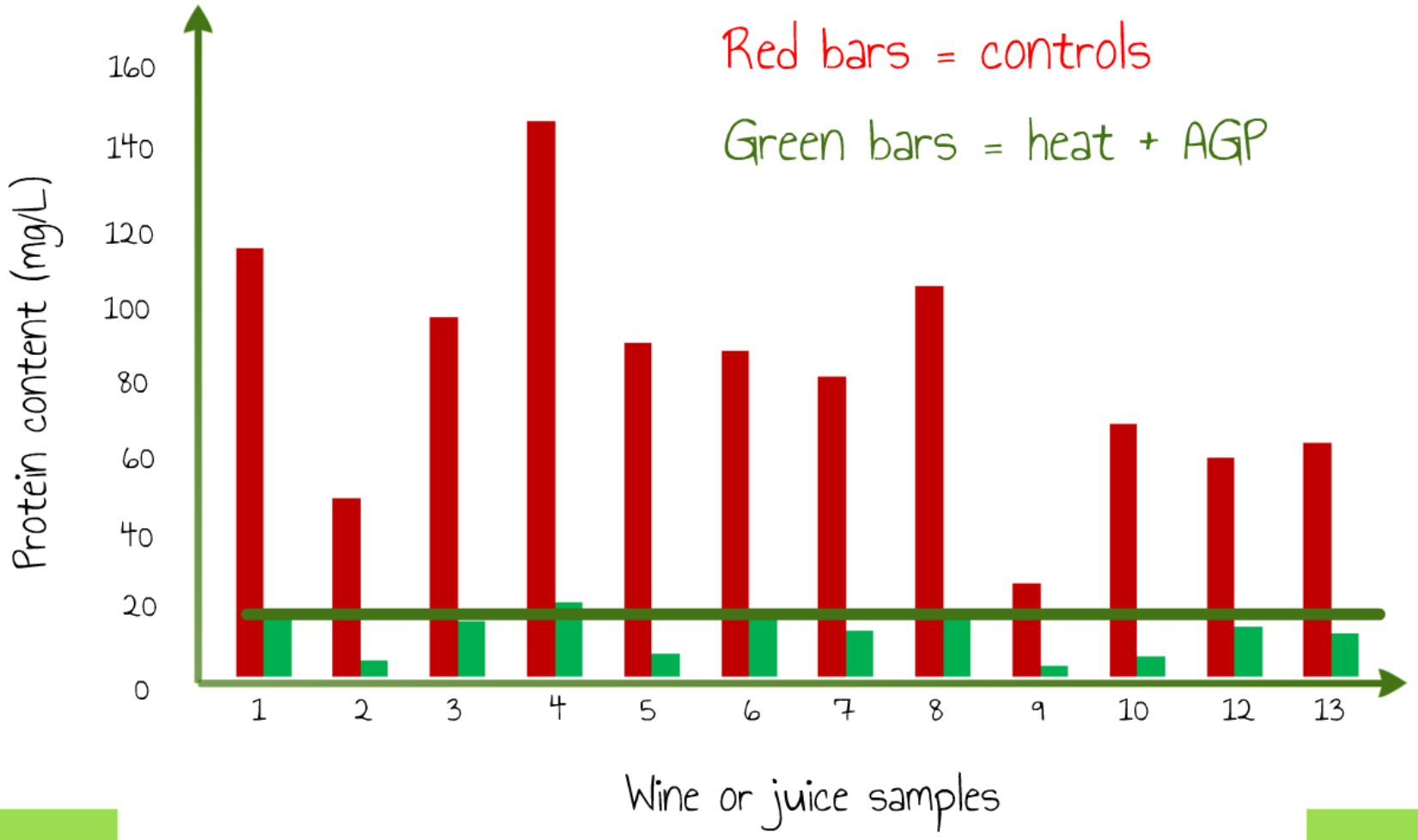


Effect on juice protein profile



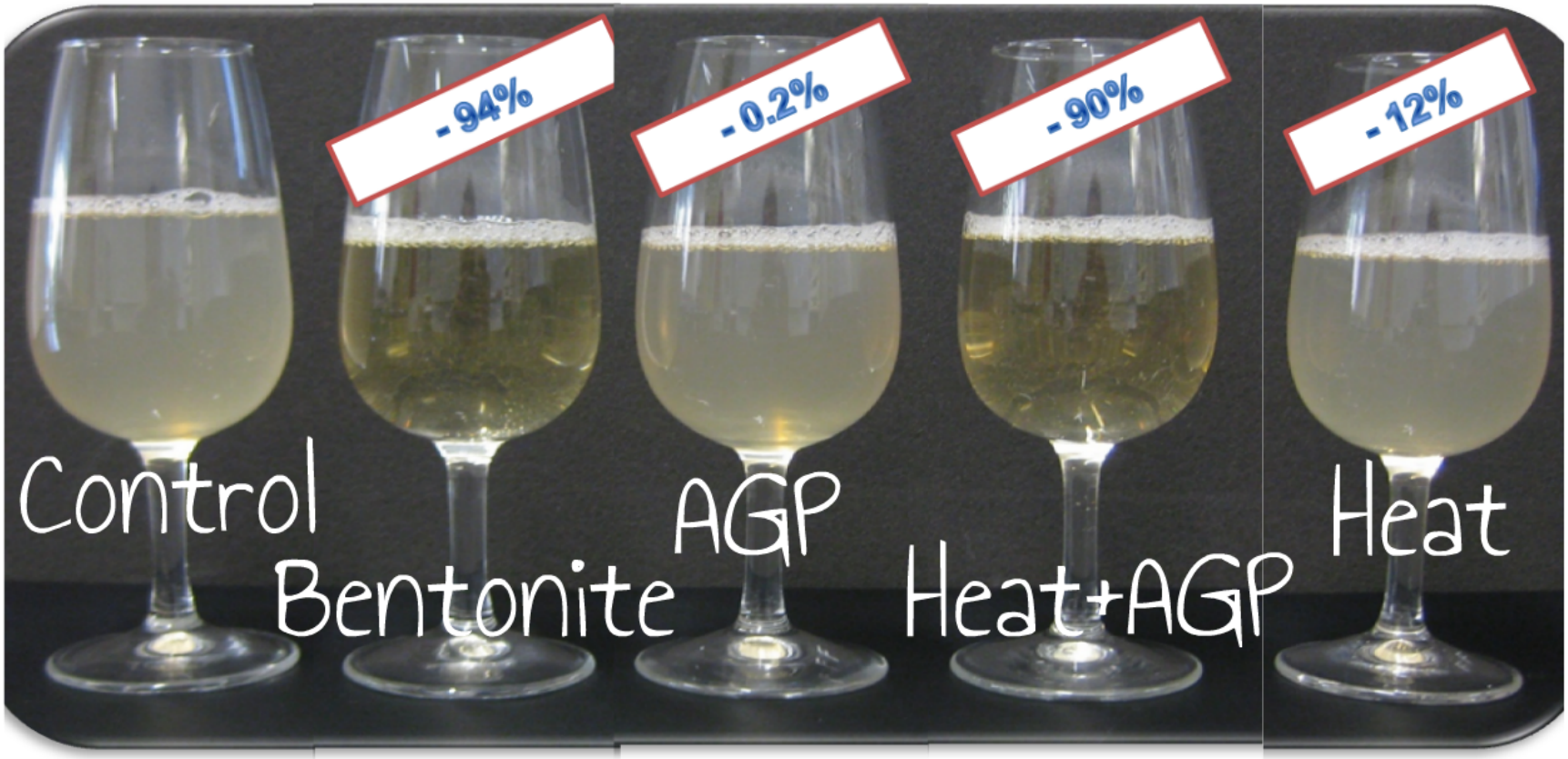
Sauvignon blanc juice V2011

Effect on wine protein content

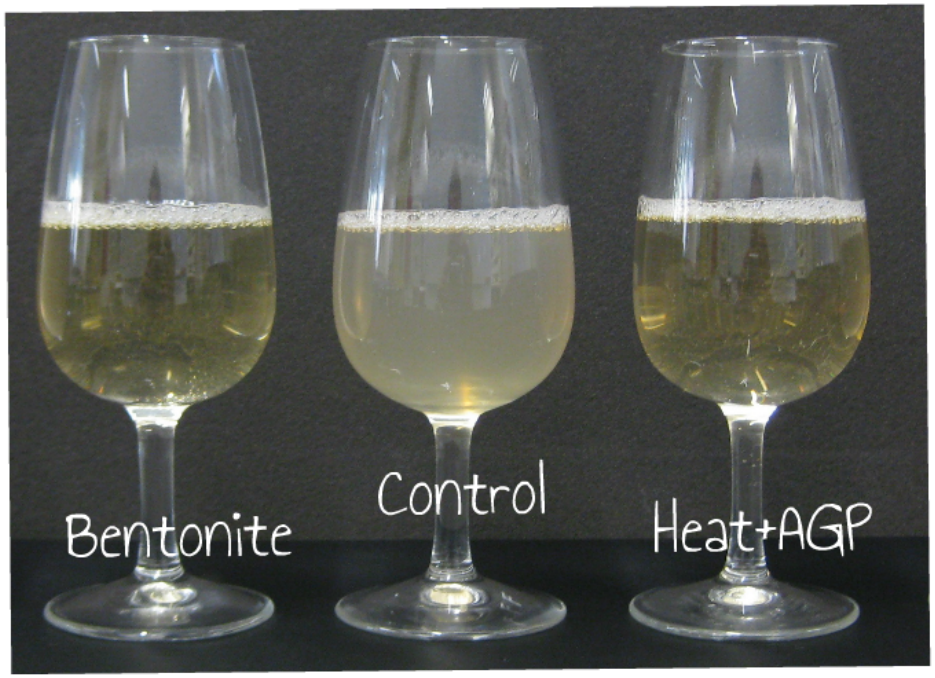


Effect on heat stability

Heat test 2h @ 80C, 2h ice



Effect on heat stability



AGP treated wines still haze free after 1 years storage at 15C and their protein content unchanged

pH	−	=	=
EtOH	=	=	=
SO2	=	=	=
T.A.	−	=	=
Colour	=	=	=

Sensory effect

UNSTABLE -
non commercial

Different

≠

STABLE -
Industry Standard

Not different

=

STABLE -
new treatment

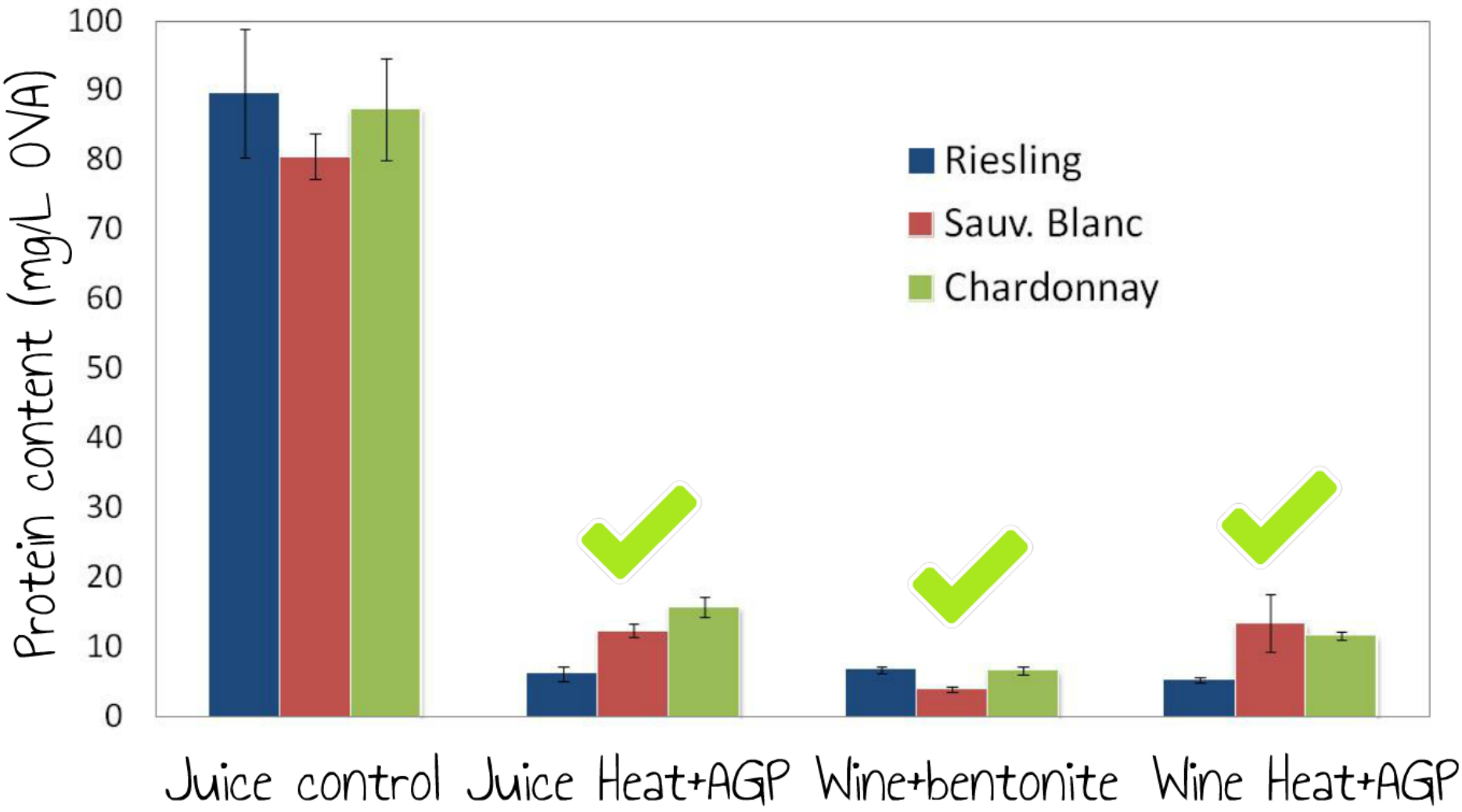
triangle test, 47 judges, $P < 0.01$

Vintage scale up

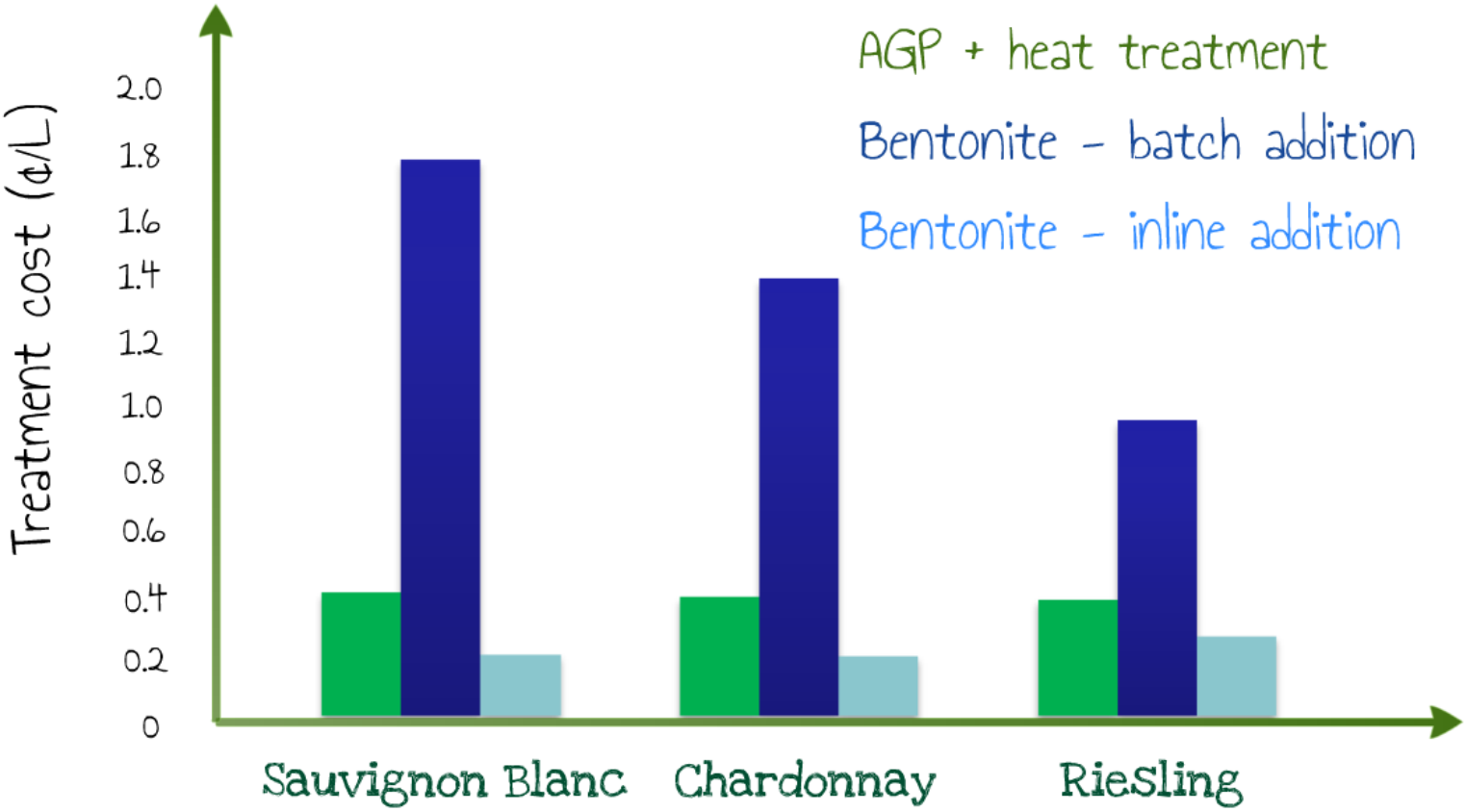
- Two industry partners
- Three juices (Riesling, Chardonnay, Sauvignon blanc)
- 5000L scale
- Same conditions (~ 1 minute, ~ 70°C, 15 mg/L Proctase)

- Two treatments:
  a. Unheated control + Bentonite on finished wine (industry standard)
  b. Heated juice + AGP

Protein content



Is this approach cost competitive?



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

Is it an allowed process?

- Enzymes of the same origin (Aspergillus niger var. macrosporus) are already approved as winemaking additives.
- AWRI application with FSANZ to have AGP approved for winemaking was succesfull
- Currently with OIV to receive full approval but already accepted by major markets

Conclusions

- AGP works! Effective degradation of the haze forming proteins when combined with flash pasteurization
- AGP does not affect the physicochemical and sensorial wine characteristic
- AGP treatments are cost competitive with traditional stabilisation techniques (no lees produced)

Some practical advantages for this treatment

- Fits well with winemaking operations (cold stabilization/ beginning of fermentation)
- Juice pasteurization inactivates laccase
- Stabilizing before fermentation allows to retain yeast fermentation products
- Stable proteins left in the juice with benefits for foam (sparkling wines) and body/structure in general
- Potential to unlock glycosilated flavour compounds

Other promising enzymes

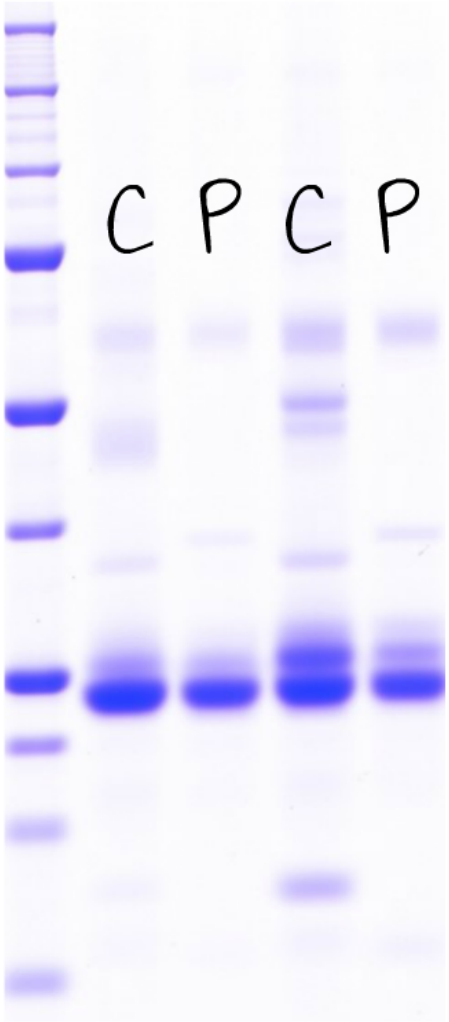
- BcAP8 (Van Sluyter et al JAFC 2013)
- Native Cysteine and Serine Proteases (Van Sluyter et al, Unpublished)
- Proteases from *Saccharomyces cerevisiae* (Younes et al 2011, 2013)

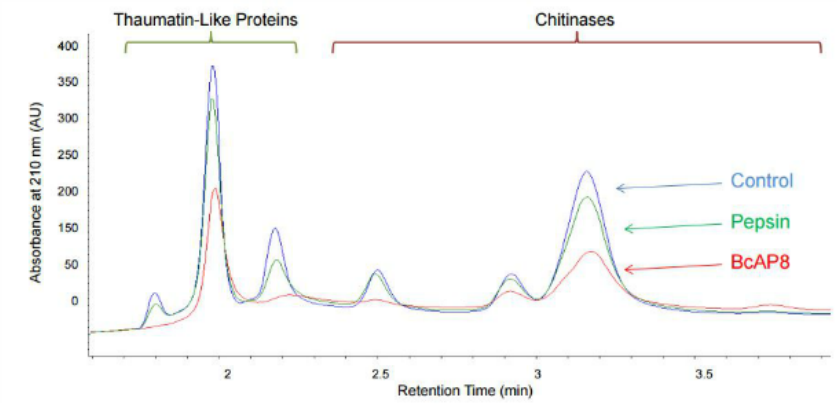
Proteases BcAP8

5 mg/L proteases during ferment

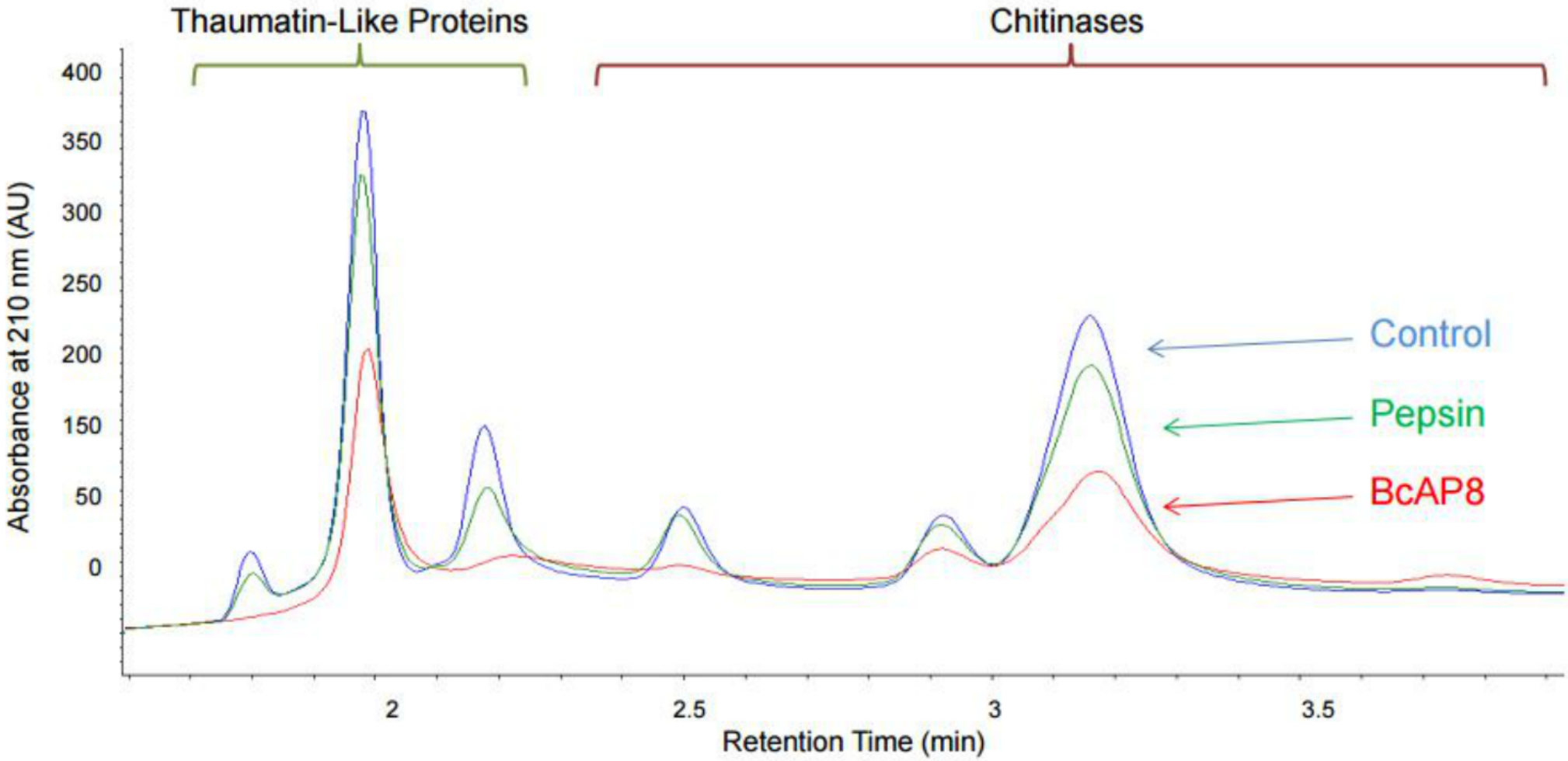
Proteases BcAP8

5 mg/L proteases during fermentation





Van Sluyter et al 2013


Thaumatin-Like Proteins
Chitinases
Absorbance at 210 nm (AU)
400
350
300
250
200
150
50
0
2
2.5
3
3.5
Retention Time (min)
Control
Pepsin
BcAP8

In general

Except AGP today there are no viable alternatives to bentonite because:

- less efficient than bentonite in removing proteins
- Often more expensive
- Longer treatment time
- Difficulties to scale up from lab trial to winery
- Possible negative effects from a sensory point of view

BUT...

- Carrageenan and Zirconia are promising
- Different enxzymes being studied for their use in fermentation

Thank you for your attention

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Managing the risk of protein haze formation in wines

Dr Matteo Marangon

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