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l'œnologie par nature

Enzymes: Natural Tools for the Modern Winemaker

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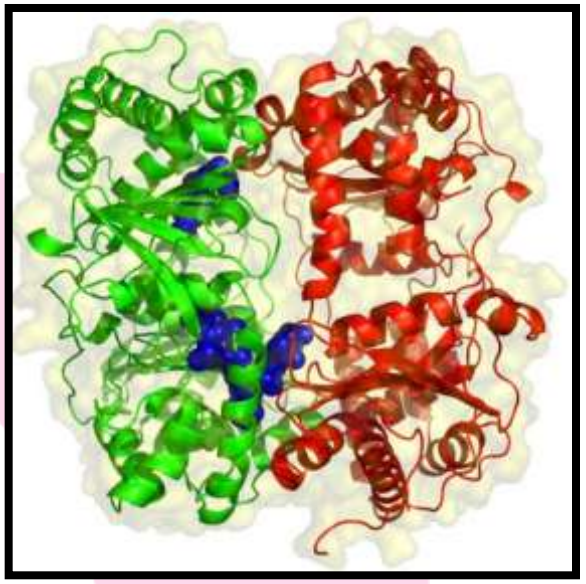
Indiana Presentation 2009

Tools for Winemakers

- Yeast and Bacteria
- Enzymes
- Nutrients
- Tannins
- Fining
- Filtration
- Stabilization

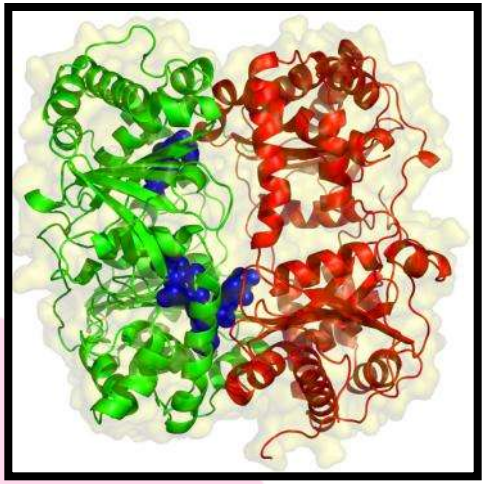
What is an Enzyme?

- A complex protein produced by living cells that promotes specific biochemical reaction by acting as a catalyst



- A tool that a winemaker can use to accomplish specific tasks at a specific time

What are Enological Enzymes?

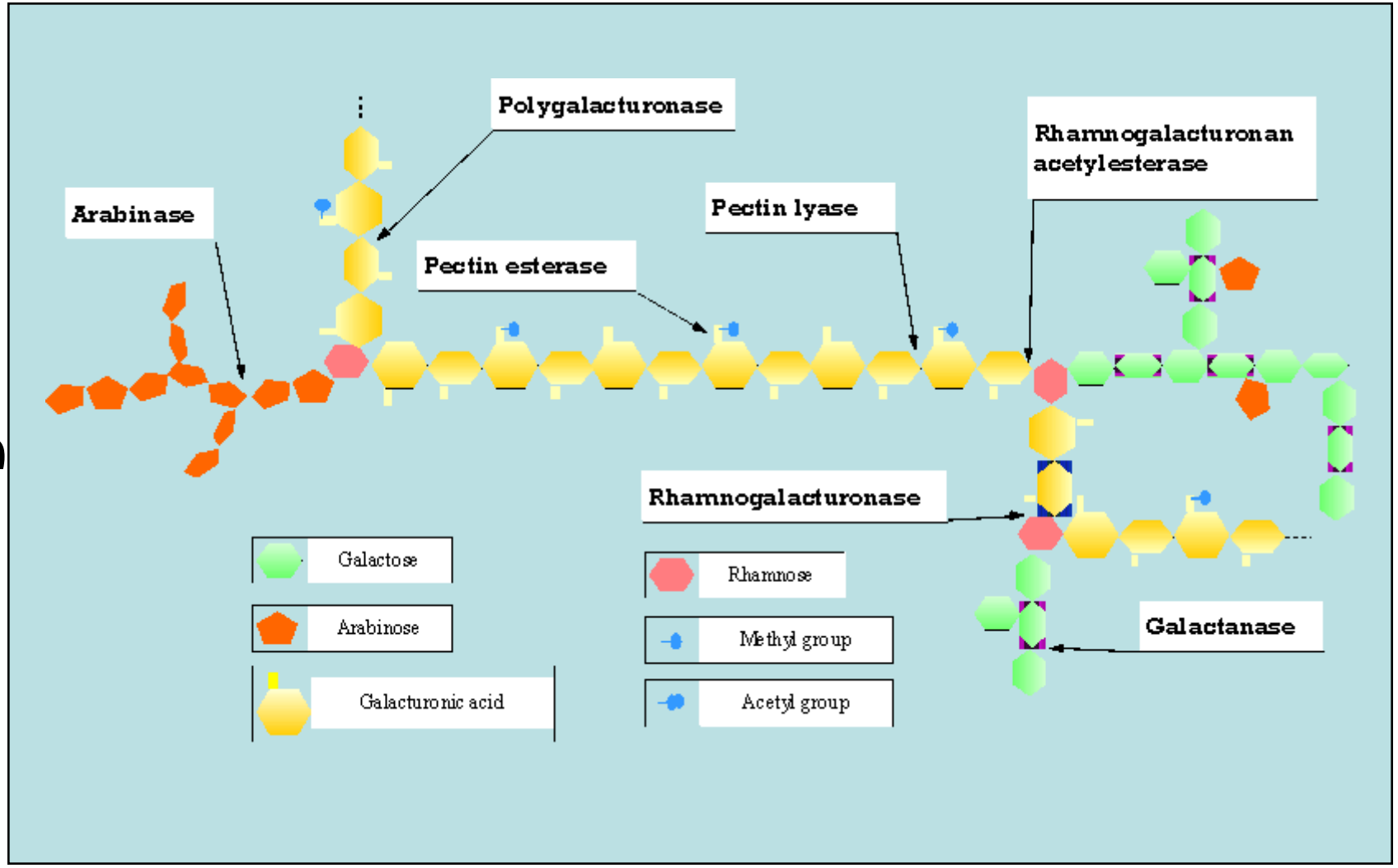


- Most commercial enzymes are a mixture of many different enzymes which typically contain:
  - Pectin esterase
  - Polygalacturonase
  - Pectin Lyase
  - Hemicellulase
  - Cellulase
- Many commercial enzymes contain other side activities that can be detrimental
  - Anthocyanase
  - Cinnamyl esterase
- Early enzyme preparations were developed for the fruit (apple) industry for one sole purpose- increase yield
- Derived from Aspergillis niger – a fungus

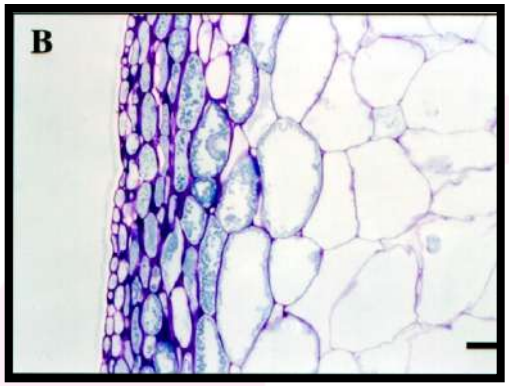
Enzymes as Tools

- Naturally present in grapes and yeast
- Designed to break down cell walls and pectinase quickly
- Cuts at very precise and specific points
- Designed to make winemaking easier and more predictable
- Each preparation designed for a specific task
- You must understand what your specific need is

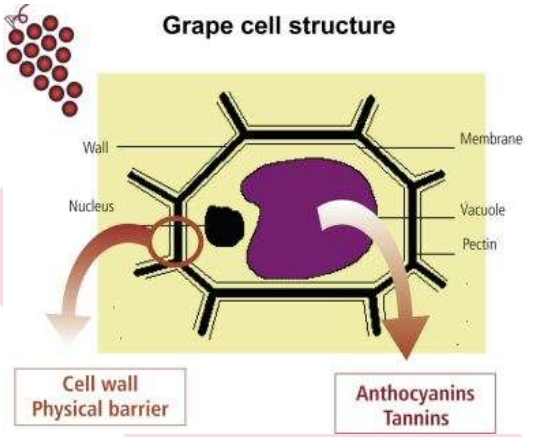
Enzymatic hydrolysis of pectin substances: Depectinization

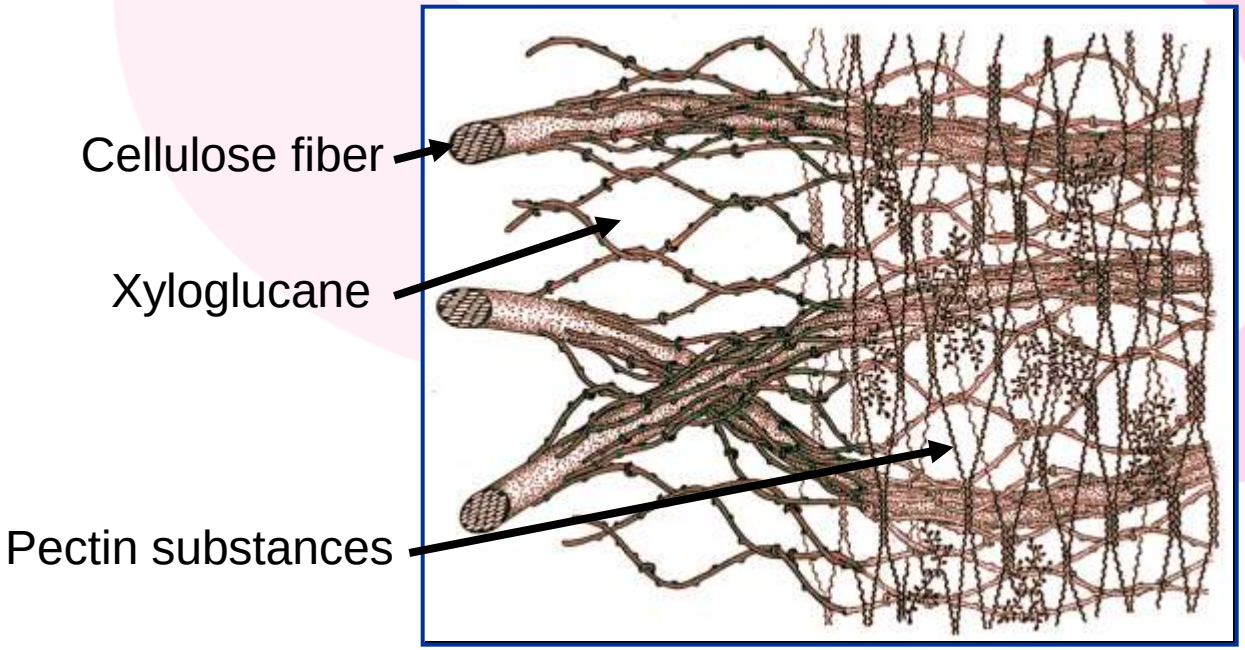


Enological enzymes work on grape skin cells



Grape skin cells





Grape skin cell wall structure

Why use enzymes?

- Originally used to increase yield (Bulk=Cheap)
  - Extremely effective but can harm wine quality
  - Very effective on Labrusca varieties
  - Also good for hybrid varieties
- Increase clarification
- Improve filterability
- Increase color and tannin extraction
- Improve complexity, mouthfeel and stability
- Release aroma compounds
- Break down yeast cell walls

Purification and Side-activities

- Bulk enzymes contain many “activities”
- Many activities can be detrimental
  - Cinnamyl esterase can lead to phenolic characters (band-aid, pharmaceutical) (4-vinyl phenol)
  - Anthocyanase can lead to color break down
  - Too much activity can lead to complete degradation of skins and excess sludge
- Purification can remove these bad side-activities
- High quality enzyme preparations designed to be gentle and specific for grape extraction

White winemaking

"In the winemaking of the dry white wines, important choices are made before alcoholic fermentation; then, there are no possible corrections or adjustments. Once alcoholic fermentation has started, potentially the taste of white wines are already largely given."

Extract from Traité d'œnologie 1998.

Optimal pressing conditions

(Traité d'œnologie, 1998)

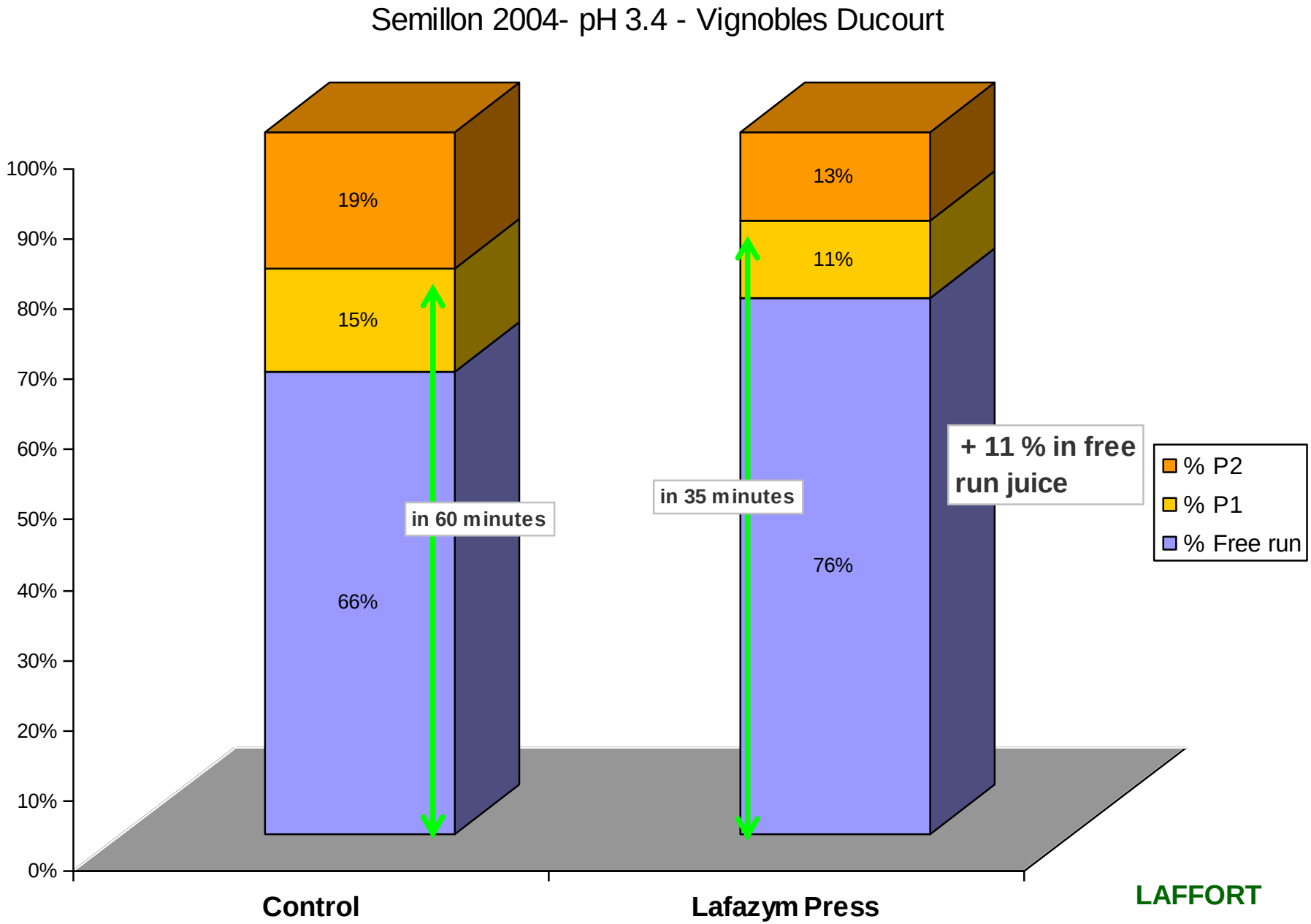
A well-planned extraction should limit:

- the phenomena of oxidation
- the extraction of phenolic compounds from the skin
- a pH increase mainly linked to the extraction of potassium from the solid parts of the grape

Effects of high quality enzyme preparation

- Improve the yield extraction of free run juice
- Optimize pressing (lower pressure and shorter cycles)
- Limit skin tearing and harsh treatment
- Efficient at low temperature
- Very little variation in pH
- Facilitate settling, decrease turbidity
- Limit aromatic deviations (volatile phenols) and preserve the aromatic freshness

Juice yields and time of pressing



Aroma results

Aromatic profile analysis of Sauvignon Blanc wines and determination of the chromatic characteristics – Trial New Zealand

Enzyme trial, Sauvignon Blanc ,NZ : aromatic profile

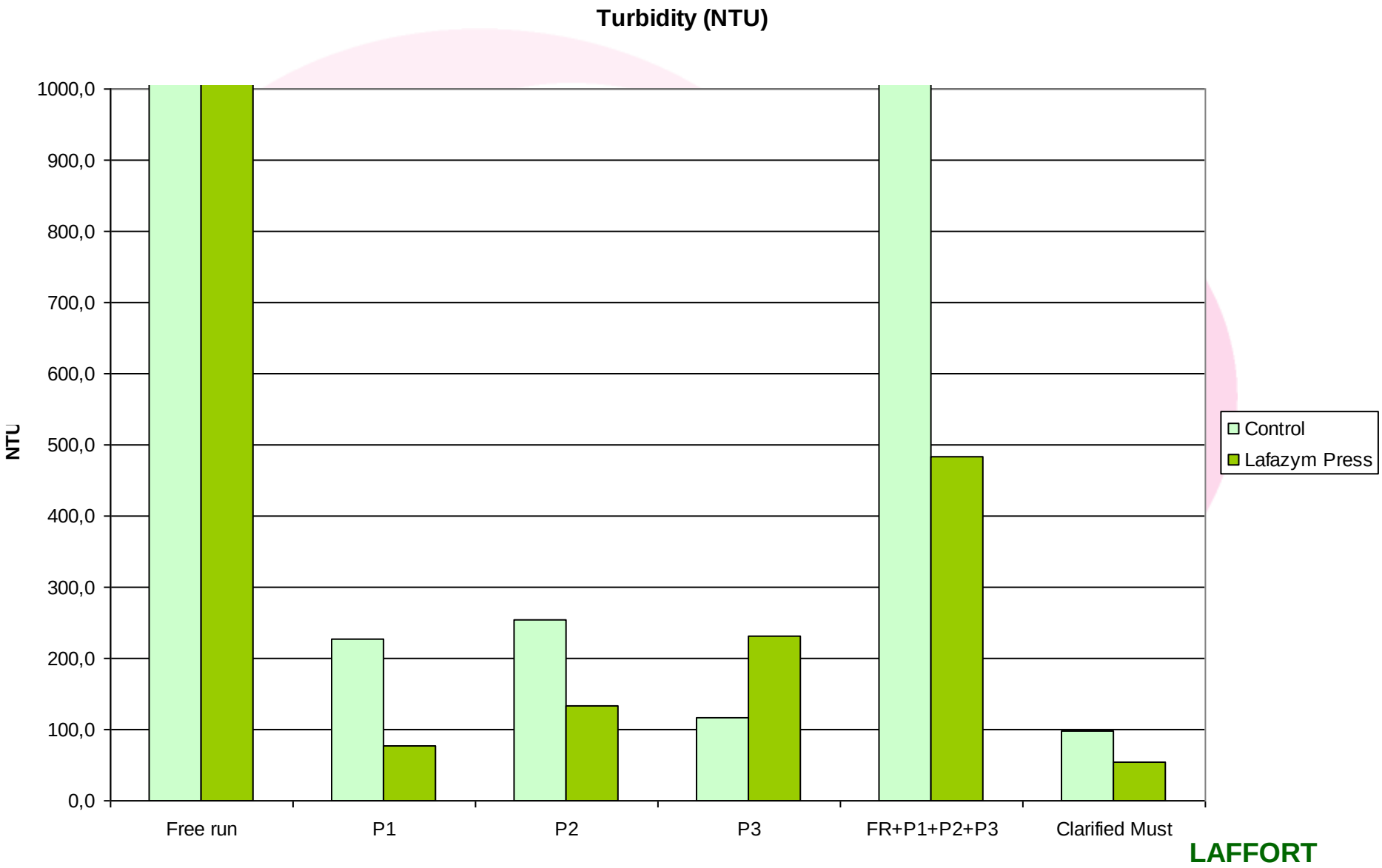
	[3MH] in ng/L grapefruit, passion fruit	[A3MH] in ng/L Passion fruit	[4MMP] in ng/L Box tree, broom	[IBMP] in ng/L Green pepper
Lafazym Press	**870**	**126**	**nd**	**3**
Enzyme B	**598**	**79**	**nd**	**2**

Lafazym Press releases more aromas than the control, enzyme B and preserves analytical characteristics.

Enzyme trial , Sauvignon Blanc ,NZ

	Lafazym Press	Enzyme B
Total acidity of tartaric acid)	7,73	7,73
pH	3,24	3,24
Malic acid (g/L)	3,67	3,6
Volatile acidity (g/L acetic acid)	0,22	0,29
Free SO_2 (mg/L)	63	72
Total SO_2 (mg/L)	166	162
Optic density at 420 nm	0,084	0,093
Optic density at 520 nm	0,024	0,037
Optic density at 620 nm	0,011	0,025
S 420+520+620	0,12	0,16

Lafazym Press trial: Turbidity



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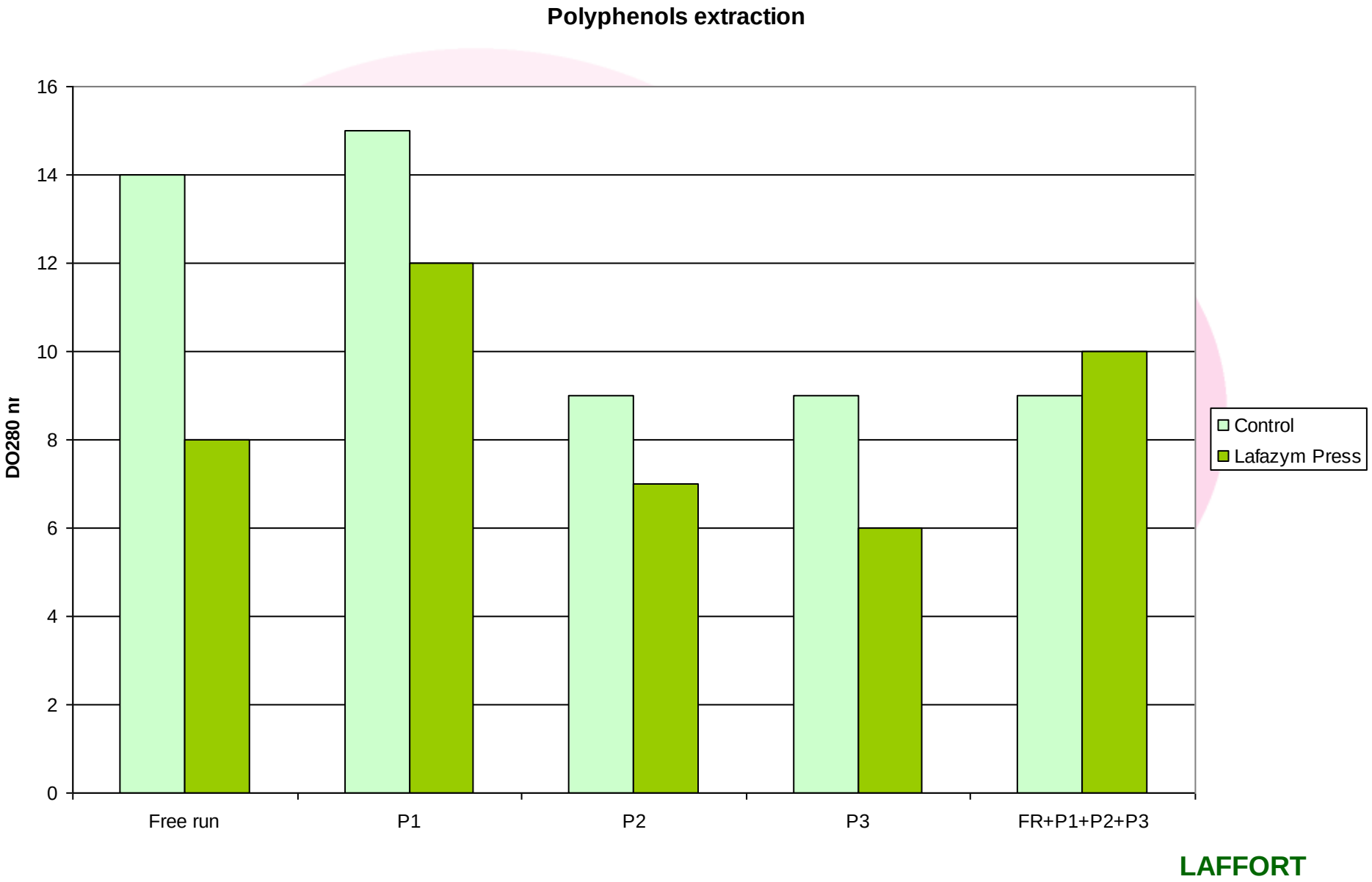
Lafazym Press trial: Phenolics



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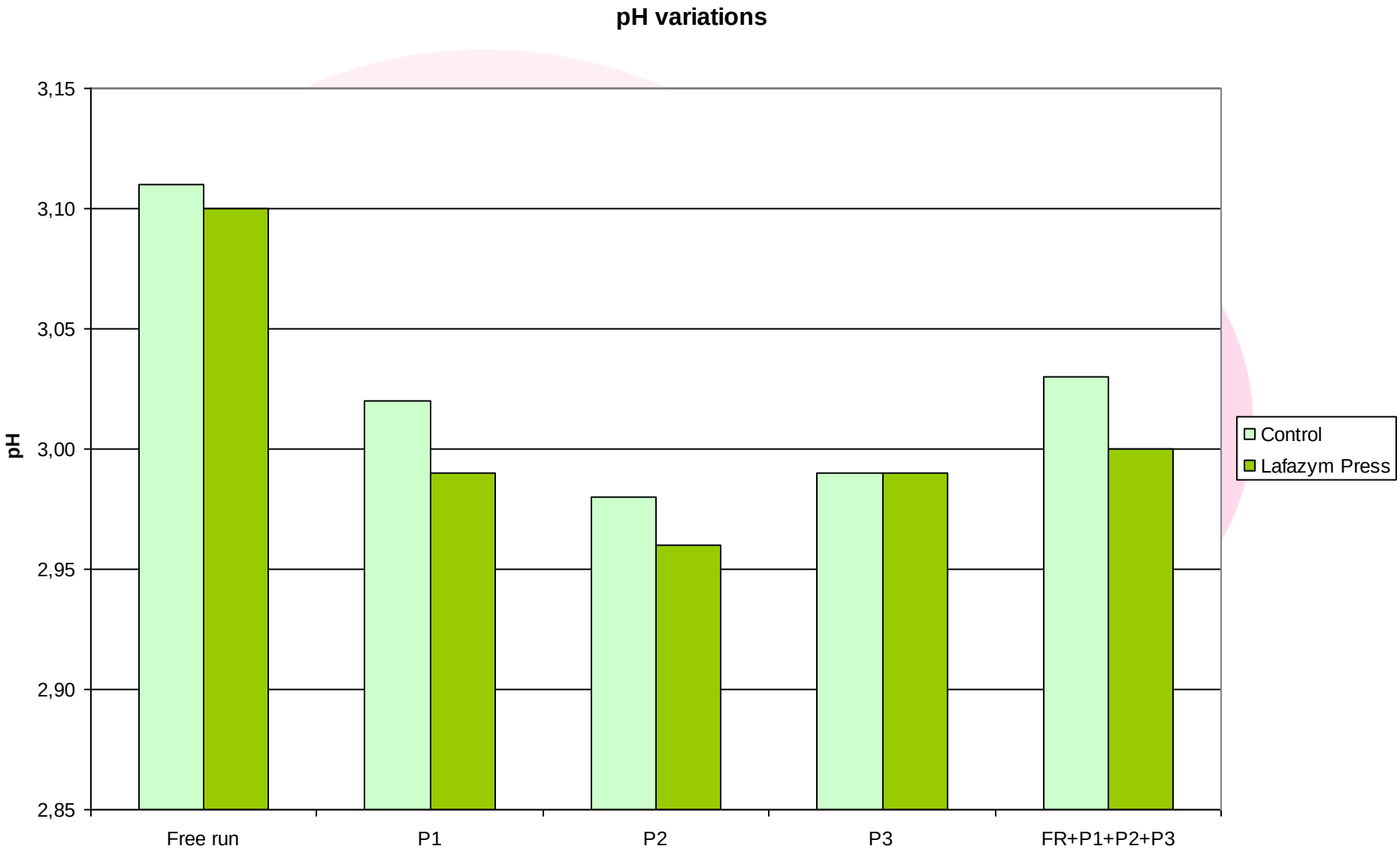
Lafazym Press trial: pH variations



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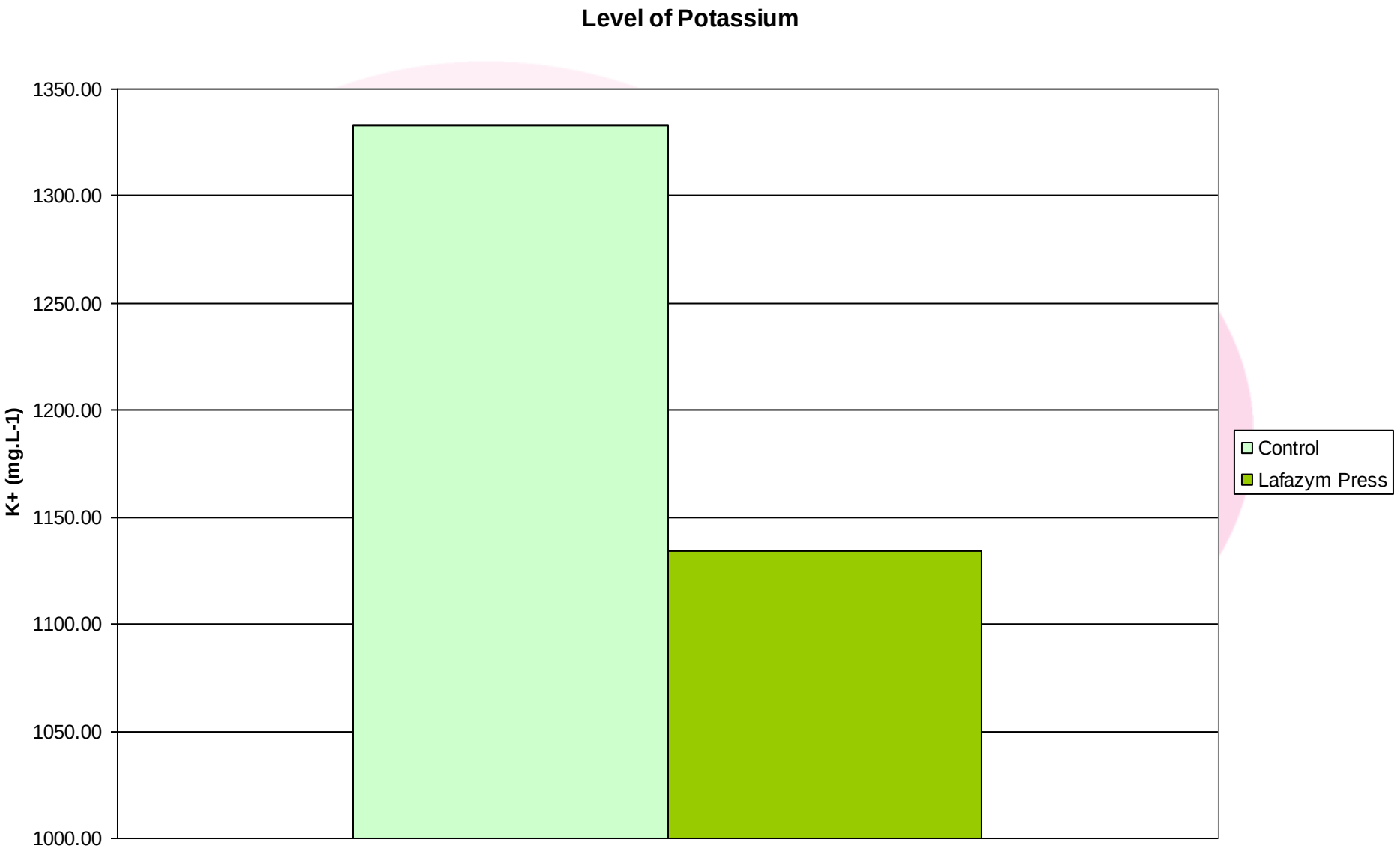
Lafazym Press trial: K+ extraction



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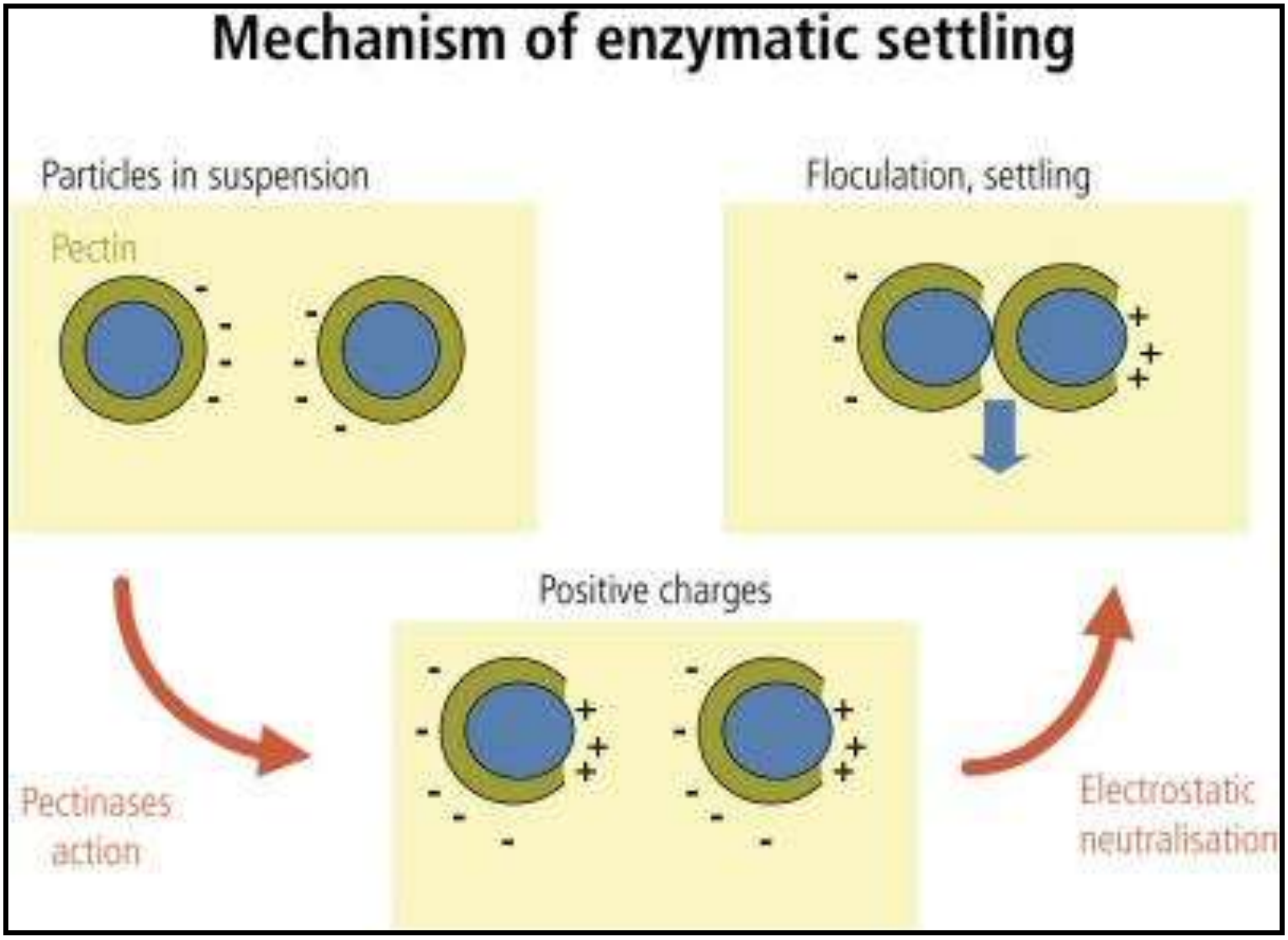
White wine extraction

- Improved aroma extraction
  - Lafazym Press significantly improves aroma extraction
- Improved yields without degrading quality
- Improved clarity in juice
- Improved filtration and settling
- Enzyme designed to sprinkle on the skins
- Improves overall quality

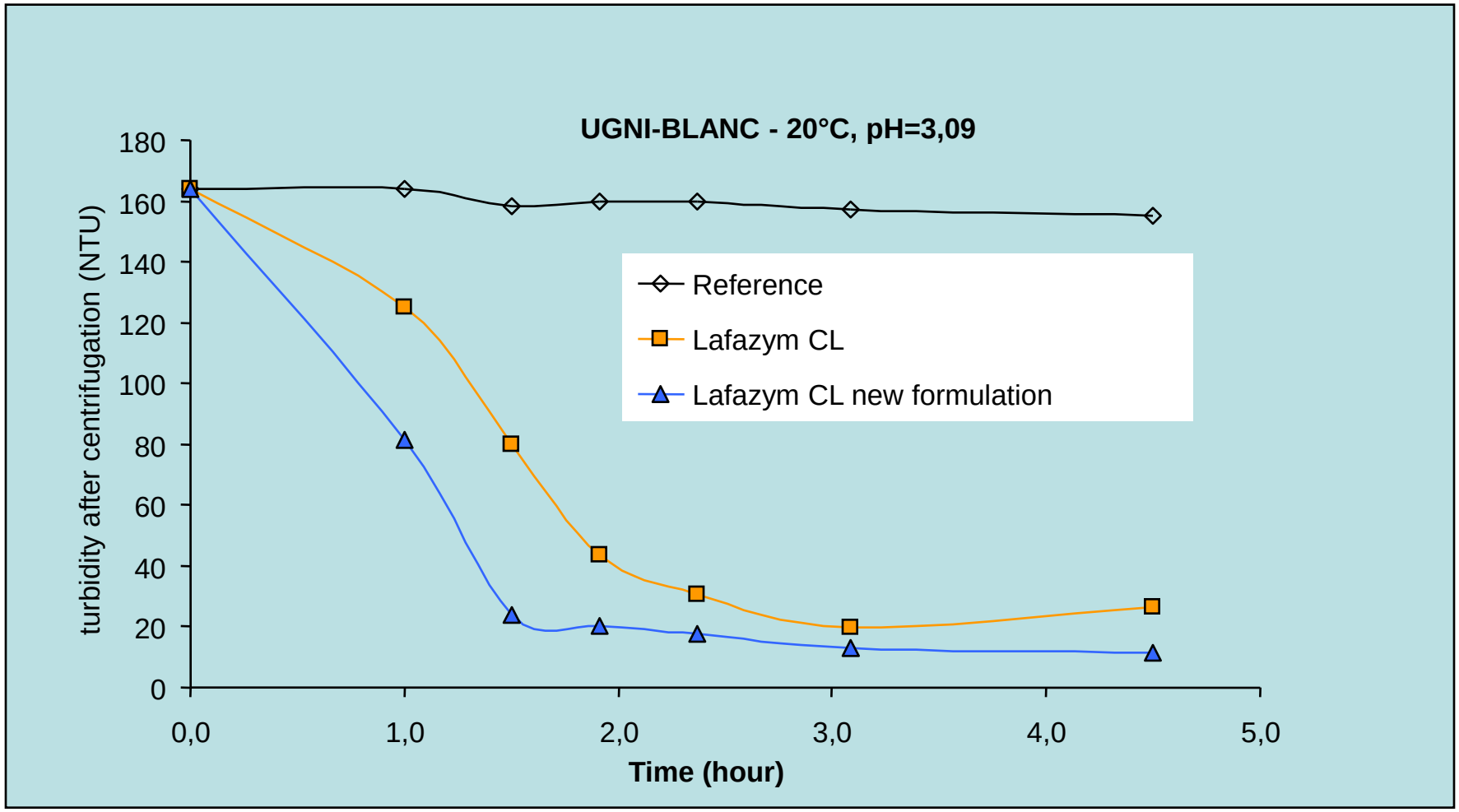
Clarification

- For white and rose wines
- Goal is to break down pectinase as quickly as possible to get maximum clear juice
  - Generally cool so enzyme needs to work in this environment
  - Lafazym CL designed for this purpose
  - Needs to work quickly
- Do not over clarify
- Improves quality of wine
- Add to press pan or filling tank
- Do not use with bentonite

HOW DOES THIS WORK?



Clarification



Why use enzymes in red wine

- Enzymes function to break up skin cells quickly
  - Tannins and anthocyanins are contained in the skin cells
  - Anthocyanins are water soluble and go into to solution quickly
  - Tannins needed to prevent oxidation of anthocyanins- but typically need alcohol to be dissolved
- Improved pressing
  - Improves residual sugar
  - Improves free run yield
- Improves color, tannin extraction and stability
- With purified enzymes there is less phenolic acid precursors for *Brettanomyces*
- Wine is more clear and settles faster

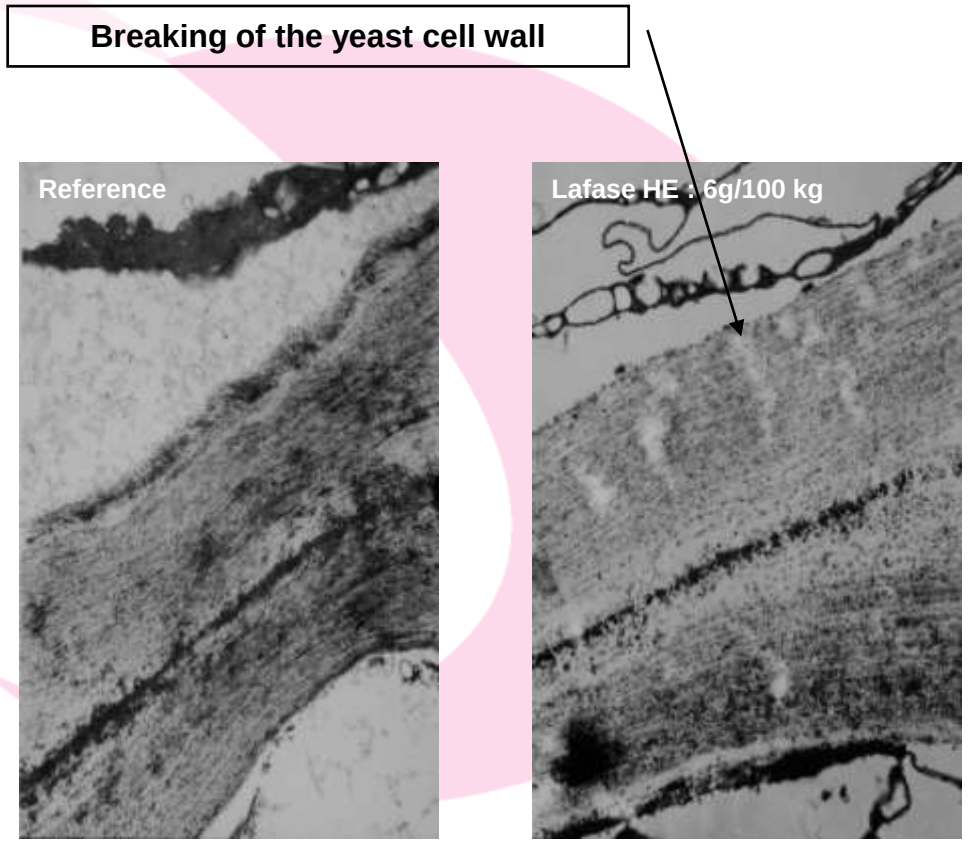
Red wine extraction and maceration

- Selection, usage and dose depends on varietal, quality of fruit, target wine
  - Enzymes do not add color
  - Enzymes do not add tannins
- Take into consideration what your mechanical plans for the wine
  - Lower dose if thin skins
  - Lower dose if using pump-overs and have more time
  - Increase dose if colder temp or want extraction in less time
- Use high quality enzymes to improve quality and make downstream winemaking easier
  - Spray on skins as you de-stem
  - Consider using tannin addition as the effect is synergistic
- Do not add SO_2 directly into enzymes
- Do not add bentonite

Enzyme Activities: Lafase HE Grand Cru

Enzymatic actions in cells: Extraction

Lafase drills hole in the cell wall to create a gentle extraction of the most interesting components of the cell: free complexes of tannins in vacuole, tannins bounded to polysaccharides, polysaccharides, … at the earlier stage of the vinification



Extraction process with Lafase HE Grand cru

Harvest parameters

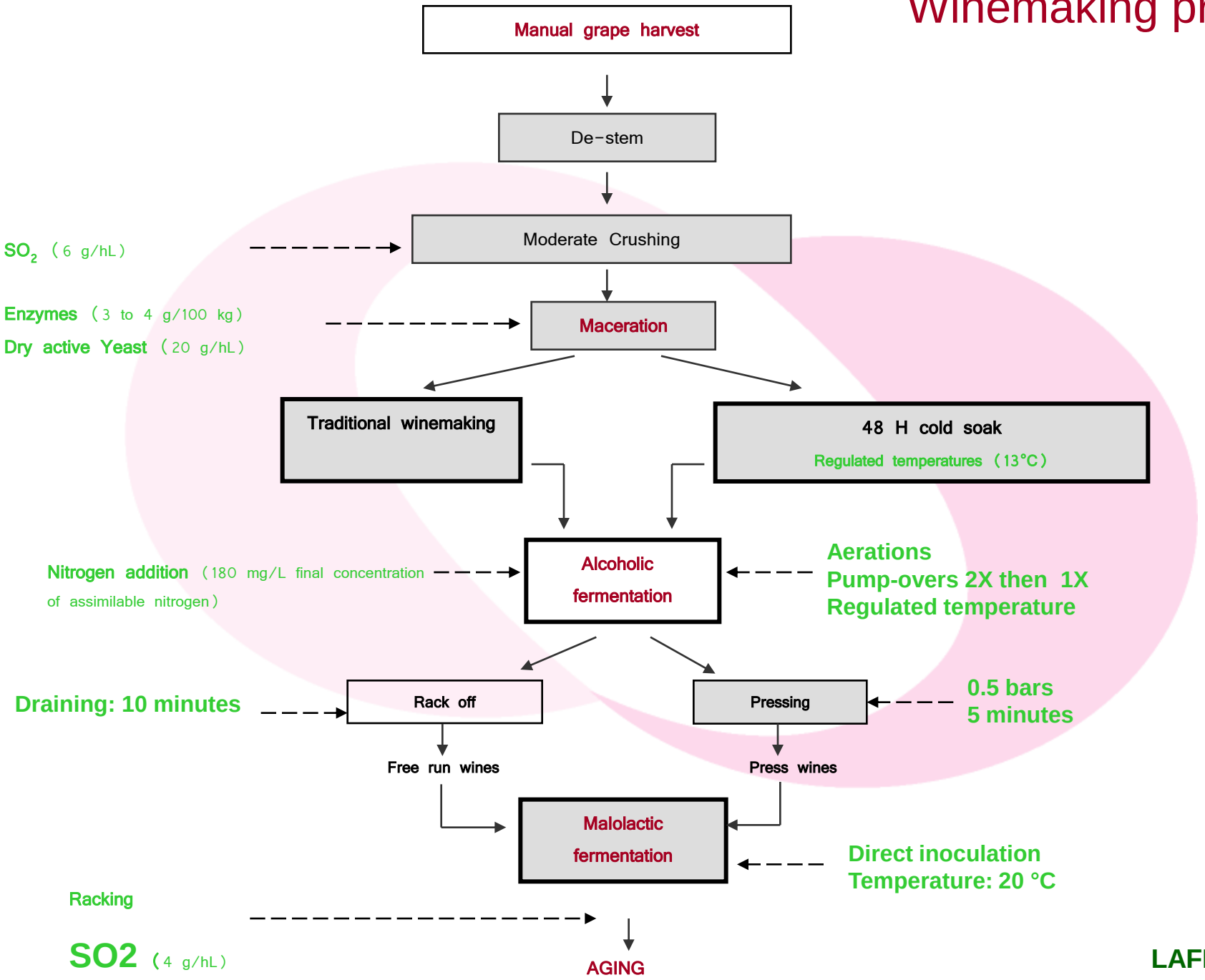
Merlot

Manual grape harvest	Reducing sugar (g/L)	Total acidity H_2SO_4 (g/L)	pH	A_{280nm}	Anthocyanin at pH3.2 (mg/L)	Malic acid (g/L)
Sept 28th	215 (21.5 BRIX)	2.8	3.5	56.8	852	1.89

Cabernet Sauvignon

Manual grape harvest	Reducing sugar (g/L)	Total acidity H_2SO_4 (g/L)	pH	A_{280nm}	Anthocyanin at pH3.2 (mg/L)	Malic acid (g/L)
Oct 14th	218 (21.8 BRIX)	4.01	3.4	57.0	1057	2.71

Winemaking protocol



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Comparison of the pomace (Merlot)

Control

Lafase HE Grand Cru
4 g/100 kg

Under the same mechanical actions, the pomace of the treated must is dryer and more extracted than the one of the control.

Colorimetric analysis Merlot

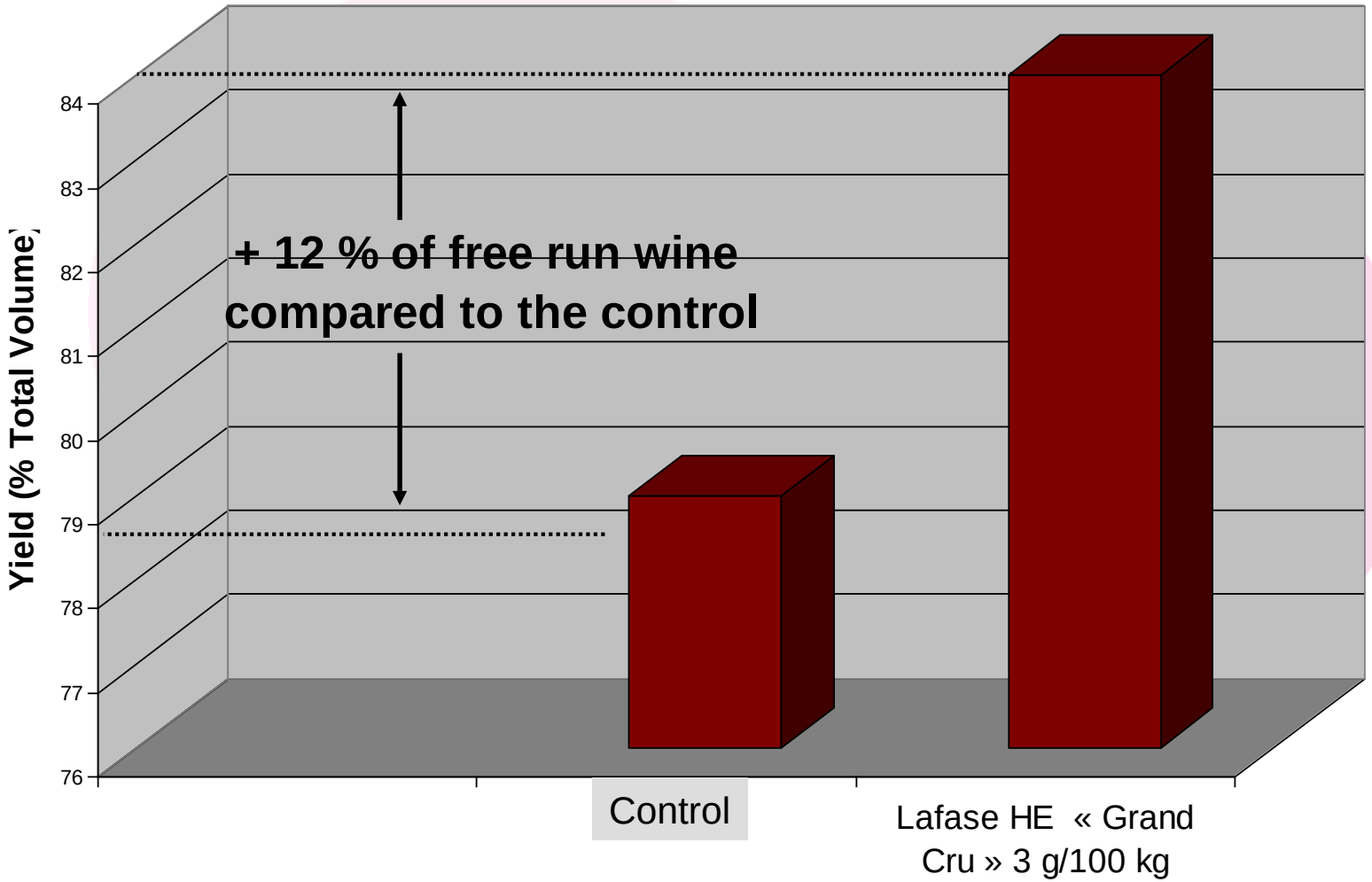
Samples	Enzyme	Cold soak	2 days maceration		5 days maceration		8 days maceration	
			Color intensity	A280	Color intensity	A280	Color intensity	A280
Control	0	Yes	0.04	10.9	0.81	24.6	0.99	46.6
Lafase HE « Grand Cru »	4 g/100 kg	Yes	0.04	**13.3**	1.54	**31.1**	1.41	**51.4**
Lasase HE « Grand Cru »	4 g/100kg	No	0.06	11.7	1.30	25.8	1.54	**51.4**

The modified color intensity and the total phenolic compounds were measured regularly during winemaking. Enzymes allow more extraction of color than the natural extraction.

Volumes of free run and press wines

	Samples	Free run wine (% total Vol)	Press wine (% total Vol)
MERLOT	Control	84.5	15.5
	Lafase HE Grand Cru 4 g/100 kg (no cold soak)	**89.2**	10.8
	Lafase HE Grand Cru 4 g/100 kg (with cold soak)	87.7	12.3
CABERNET	Control	79	21
	Lafase HE Grand Cru 3 g/100 kg (no cold soak)	**84**	15
	Lafase HE Grand Cru 3 g/100 kg (with cold soak)	82	18

Comparison of free run wine yields
Cabernet Sauvignon



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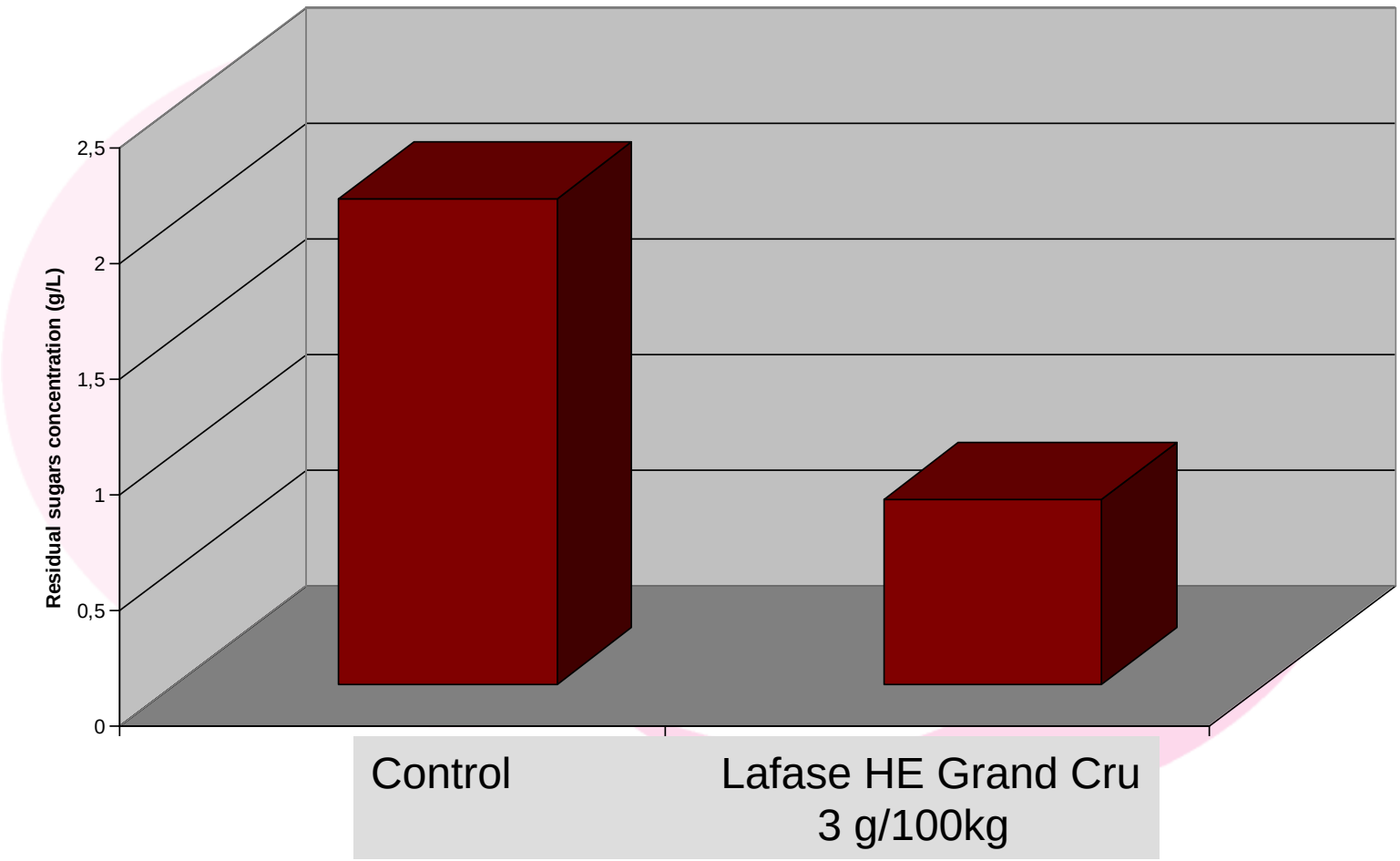
Residual sugar in press wines
Cabernet Sauvignon



Press wines with enzymes contained less residual sugar (0.8 g/L) than those with no enzymes (2.1 g/L)

Polyphenolic profile - Merlot 2004

Lafase HE Grand Cru
4 g/100kg,
no maceration

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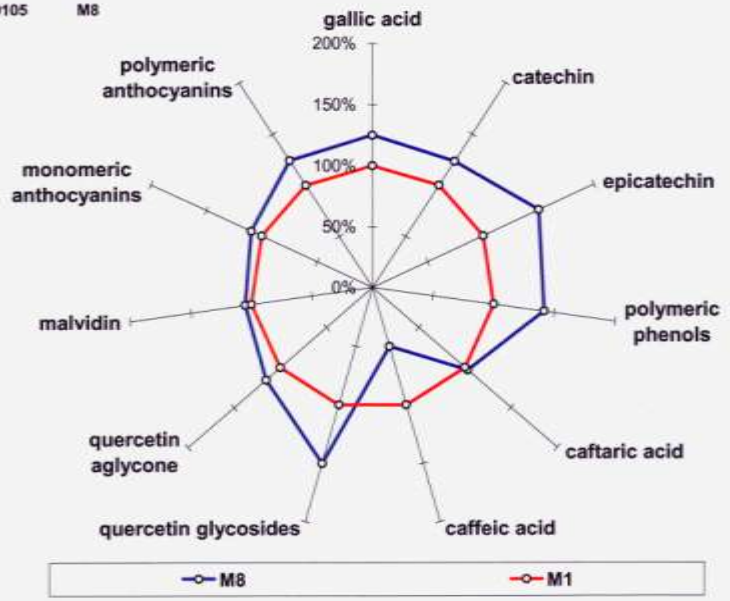
REPORT # 193247 SUPPLEMENT

Marie Laure Murat
Sarco
PO Box 40
Bordeaux France 33015

Samples Received
18 January 2005

Analyses Reported
20 January 2005

501180105 **M8**



gallic acid (mg/l)	10	total anthocyanins (mg/l)	527
catechin (mg/l)	37	malvidin glucoside (mg/l)	232
epicatechin (mg/l)	12	monomeric anthocyanins (mg/l)	481
polymeric phenols (mg/l)	614	polymeric anthocyanins (mg/l)	46
caftaric acid (mg/l)	31	quercetin glycosides (mg/l)	24
caffeic acid (mg/l)	1	quercetin aglycone (mg/l)	15
catechin:epicatechin ratio	3.08	catechin:polymeric phenols ratio	0.06

Comments: The reference for the radar plot is sample 501180104.

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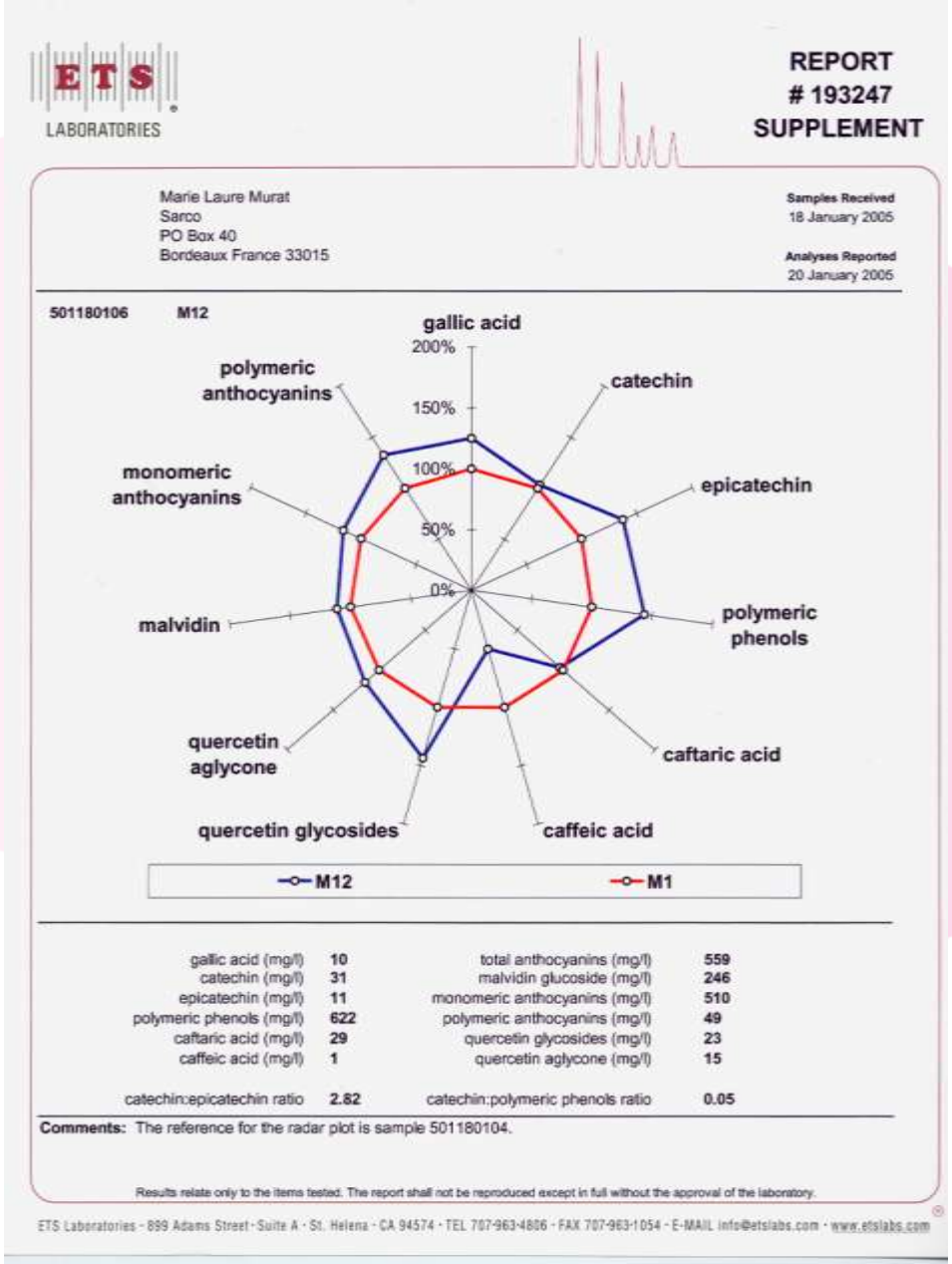
Polyphenolic profile - Merlot 2004

Lafase HE Grand Cru
4 g/100kg with cold soak



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REPORT # 193247 SUPPLEMENT

Marie Laure Murat
Sarco
PO Box 40
Bordeaux France 33015

Samples Received 18 January 2005

Analyses Reported 20 January 2005

501180106 **M12**

gallic acid (mg/l)	10	total anthocyanins (mg/l)	559
catechin (mg/l)	31	malvidin glucoside (mg/l)	246
epicatechin (mg/l)	11	monomeric anthocyanins (mg/l)	510
polymeric phenols (mg/l)	622	polymeric anthocyanins (mg/l)	49
caftaric acid (mg/l)	29	quercetin glycosides (mg/l)	23
caffeic acid (mg/l)	1	quercetin aglycone (mg/l)	15
catechin:epicatechin ratio	2.82	catechin:polymeric phenols ratio	0.05

Comments: The reference for the radar plot is sample 501180104.

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Other Enzymes

- β-glucosidase
  - Cleaves glucose from terpenes in wine to release aroma- *Gewurtztraminer, Traminette, Riesling*
  - Does not work as well in juice (glucose is inhibitory)
- Proteases
  - Currently none that work at wine pH

Other Enzymes

- β - 1,3 glucanase for grapes
  - Botrytis produces glucanase
  - Certain grape varietals have increased glucanase
  - Too much glucanase degrades filtration
  - Improves filtration and settling
- β - 1,3 glucanase for lees
  - Lees (dead yeast) cell walls made from glucanase
  - Lees aging improves mouth feel and stability
  - Speeds up lees aging and need less stirring

Caution

- β - 1,4 glucanase does not work in grapes
  - Usually much cheaper (use in beer)

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Thanks for your attention