

# The relationship between enzyme treatment and polysaccharide extraction in winemaking, and subsequent sensory effects in Cabernet Sauvignon wines.

Brock KUHLMAN<sup>1</sup> Bodil JØRGENSEN<sup>2</sup>, José L. ALEIXANDRE TUDO<sup>3,1</sup>, Wessel DU TOIT<sup>4</sup>, John P. MOORE<sup>1</sup>

<sup>1</sup>South African Grape and Wine Research Institute, Stellenbosch University, Stellenbosch, South Africa, <sup>2</sup>Plant Glycobiology, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark, <sup>3</sup>Universitat Politècnica de València, Instituto de Ingeniería de Alimentos para el Desarrollo (IIAD), Departamento de Tecnología de Alimentos, <sup>4</sup>Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa

## INTRODUCTION

**Astringency is a primary quality attribute for red wine.**

- Along with acidity, sweetness, aroma, bitterness, and minerality, the **balance of these perceptions determines the quality of the wine.**

**Astringency itself is not a well understood phenomenon;** it is possibly a directly sensed concentration of astringent molecules, a loss of lubrication, a formation of protein precipitates, or a combination.

- In model wine:
  - increasing concentration of tannin increases astringency
  - Added plant polysaccharides cause a decrease in astringency.
- In red wine
  - the simple **tannin-astringency model fails**
  - maybe due to complicated tannin populations
  - maybe due to interaction with complicated polysaccharide populations.

**Enzyme-assisted maceration is a common wine making process.**

Pectolytic enzymes are used to increase extraction of tannin, anthocyanin, and flavor metabolites.

- Little research has been done on the effect on sensory perception of astringency or bitterness of enzyme use.
- This research aims to determine if enzyme use has an effect that mimics increased ripening of grapes at vinification, or what effect it has on astringency and bitters in red wine.

## MATERIALS + METHODS

Cabernet Sauvignon grapes were harvested at ripeness levels most common in commercial wine making: 21°, 23°, and 25° brix. Each ripeness level was vinified with a control and enzyme treatment (Laffort HE Grand Cru, per manufacturer recommended usage)

Grape berry tissues and finished wines were tested using monoclonal antibodies that detect specific cell wall polysaccharides (CoMPP, comprehensive microarray polymer profiling), and wines were analyzed using gas chromatography of monosaccharides. Grape tissue and wines were analyzed for anthocyanin, condensed tannin, total phenolic index, color density, degree of galloylation, and degree of polymerization by a spectrometric method as well as phloroglucinolysis. Wine was tested for astringency and bitterness using a trained panel of people, after normalization of ethanol levels between wines.

All analysis were subjected to single or multiple-way analysis of variance using MATLAB software, p-values ≤ 0.05 were considered significant.

	spectrophotometry			phloroglucinolysis			monosaccharide analysis	sensory analysis	
	anthocyanin mg/L	tannin mg/L	TPI	tannin mg/L	mDP	% galloylation	ng/g saccharides in AIR	Astringency (0-5)	Bitterness (0-5)
harvest 2018									
control	382 ± 4 a	775 ± 13 a	48.6 ± 0.5 a	785 ± 25 a	2.5 ± 0 a	32.6 ± 0.7 a	79 ± 4.4 a	2.88 ± 0.12 a	2.45 ± 0.13 a
enzyme	423 ± 4 b	1039 ± 13 b	54.7 ± 0.5 b	1215 ± 25 b	2.8 ± 0 b	28.5 ± 0.7 b	68.4 ± 4.2 a	3.27 ± 0.12 b	2.50 ± 0.13 a
21	365 ± 5 a	912 ± 16 a	49.4 ± 0.6 a	1320 ± 31 a	2.6 ± 0.1 b	23.1 ± 0.8 a	63.6 ± 5.2 a	2.95 ± 0.12 a	2.42 ± 0.13 a
23	417 ± 5 b	868 ± 16 a	52.2 ± 0.6 b	1056 ± 31 b	2.9 ± 0.1 c	28 ± 0.8 b	70.1 ± 5.4 a	no data	no data
25	425 ± 5 b	941 ± 16 a	53.3 ± 0.6 b	624 ± 31 c	2.4 ± 0.1 a	40.5 ± 0.8 c	87.4 ± 5.2 a	3.20 ± 0.12 a	2.53 ± 0.13 a
harvest 2019									
control	502 ± 17 a	1392 ± 67 a	47.2 ± 1.7 a	824 ± 27 a	2.2 ± 0 a	36.2 ± 0.3 a	63.9 ± 8.6 a	no data	no data
enzyme	600 ± 17 b	1991 ± 67 b	58.9 ± 1.7 b	1052 ± 27 b	2.2 ± 0 a	33.8 ± 0.3 b	55 ± 8.8 a	"	"
21	523 ± 20 a	1443 ± 82 a	49 ± 2.1 a	1030 ± 32 a	2 ± 0 a	32 ± 0.4 a	66.4 ± 10.7 a	"	"
23	565 ± 20 a	1781 ± 82 b	55.5 ± 2.1 a	877 ± 33 b	2.2 ± 0 b	36 ± 0.4 b	55.2 ± 10.2 a	"	"
25	565 ± 20 a	1849 ± 82 b	54.6 ± 2.1 a	909 ± 33 b	2.4 ± 0 c	37.1 ± 0.4 b	56.8 ± 10.1 a	"	"

Table 1: Analytical chemistry results. TPI: total phenolic index; mDP: mean degree of polymerization; no sensory done on 2019 vintage because of Covid-19 restrictions.

## RESULTS + DISCUSSION

*Increasing ripeness effects in wine*

- degradation of polysaccharides, specifically homogalacturonans (HG) and arabinogalactan proteins (AGPs)
- increased tannin levels, anthocyanins concentration, and total phenolics
- increased percentage galloylation of tannin
- no effect on total polysaccharide content!

*Enzyme treatment effects in wine*

- outsized effect on polysaccharides** compared to ripeness, causing a decrease in prevalence of 50% or more for AGPs, and an almost complete loss of signal for HG, rhamnogalacturonan-I/II/III sidechains, and xyloglucan.
- increased anthocyanin and condensed tannin extraction
- reduction in galloylation of tannin
- no effect on total polysaccharide content

Sensory analysis shows **enzyme-treated wines had higher astringency** than control wines (3.27/5, compared to 2.88/5 for control, p=0.021). Neither ripeness nor enzyme treatment affected bitterness perception.

*Tannin concentration ≠ astringency*

- the tannin concentrations in this experiment do not correlate well with perceived astringency
- suggests a more complicated relationship
  - increasing mean degree of polymerization of tannin increases astringency
  - increasing % galloylation increases astringency
  - polysaccharide presence reduces astringency

The mDP of sensory-analyzed wines showed a small increase with enzyme treatment (m ~ 12%). This **increased mDP could increase astringency** of the tannin, however this shift is **counteracted by a marked decrease in galloylation of the tannins**. Wines at 21° and 25° had tannin fractions with 20% and 15% decrease respectively in tannin galloylation when treated with enzymes.

Polysaccharides common to cell walls are known to reduce the astringency of otherwise similar tannins. The **enzyme-digestion of these polysaccharides, as shown by CoMPP analysis, could reduce the normal astringency reduction**, resulting in the increased astringency seen in this experiment.

	homogalacturonans										RG-I		RG-I Side Chains			galactotriose		mannan		xyloglucans			xylans		glucans		arabinogalactans					cellulose/xyloglucan
	JIM5	JIM7	LM18	LM19	LM20	LM7	INRARU2	INRARU1	LM5	LM6	LM13	LM16	LM21	LM15	LM24	LM25	LM11	LM23	BS4002	BS4003	JIM8	JIM13	JIM15	JIM16	JIM17	LM2	MAC207	AGP	CBM3a			
Harvest 18	Control	Bx 21	0	0	0	0	0	0	0	11	0	0	0	0	0	10	1	0	0	0	42	54	0	0	0	11	0	0	0	0		
	Bx23	0	0	0	0	0	0	0	0	8	0	0	0	0	0	11	1	0	0	0	66	77	0	0	0	8	0	0	0	0		
	Bx25	0	0	0	0	0	0	1	0	13	1	0	0	0	0	13	3	0	0	0	79	88	0	0	0	6	0	0	0	0		
	Enzyme	Bx 21	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	27	0	0	0	0	0	0	0	0		
Bx23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	4	0	0	0	12	36	0	0	0	0	0	0	0	0			
Bx25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	0	0	0	12	38	0	0	0	0	0	0	0	0			
Harvest 19	Control	Bx 21	0	44	0	0	31	0	61	38	2	55	18	0	0	3	0	49	4	0	51	66	0	0	0	15	0	0	0	0		
	Bx23	0	12	0	0	1	0	31	29	0	27	3	0	0	0	0	34	7	0	0	45	56	0	0	0	7	0	0	1	0		
	Bx25	0	49	0	0	18	0	23	16	3	46	9	0	0	0	0	30	4	0	0	76	73	0	0	0	10	0	0	2	0		
	Enzyme	Bx 21	0	0	0	0	0	1	0	0	0	0	0	0	0	0	8	9	0	0	1	20	0	0	0	0	0	0	0	0		
Bx23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	4	0	0	2	16	0	0	0	0	0	0	0	1	0		
Bx25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	5	0	0	20	31	0	0	0	0	0	0	0	7	0		

Table 2: Analysis of polysaccharide epitopes via CoMPP

## REFERENCES AND ACKNOWLEDGEMENTS

