SUPPLEMENTARY DATA

S 1.1 Wine fermentation. Table S1 shows the chemical and color characteristics of the juice and the wine as determined during the harvest seasons of 2012 and 2013. The ripeness of the grapes from the two vintages showed a large difference as reflected in the Glc + Fruc (2012: 227 g/L and 2013: 265 g/L) and total acid (2012: 5.37 g/L and 2013: 4.85 g/L) values of the juice. During the 1980s various publications advised different equations for determining the optimum ripeness of wine grapes. Using the Coombe et al.[1] equation (sugar concentration [B°] x pH^2) the viticulturist should aim for a value between 200 and 270 while Du Plessis[2] proposed a sugar:acid ratio (B°: Total Acid in g/L) of four. Thus, the Pinotage harvested in 2012 meet the criteria with values of 246 and 4.2 respectively while the 2013 Pinotage would be qualified as overripe. However the current opinion of the Pinotage association (www.pinotage.co.za; April 2014) of South Africa recommends a minimum sugar level of 23 B° and that the optimum ripeness for the production of a “fuller style” Pinotage is at sugar levels between 24 and 26 B° with the total acid > 5.5 g/L and pH < 3.7. Both fermentations were fermented dry (< 5 g/L Glc + Fruc) after five and six days respectively with acceptable (0.3 – 0.6 g/L) volatile acidity levels. Furthermore, it is clear that the enzyme treatments did not significantly alter the chemical characteristics (pH, volatile acidity, total acidity, residual sugar and ethanol %) of the wine compared to the control wine of each vintage.

S 1.2 Wine color results. The wine color characteristics were measured to give an indication of the amount of anthocyanins that leached from the grape skin cells during the maceration period. This is an indirect method, in contrast to the other methods used in this article, with which to determine the extent of the permeabilization that occurred in the grape skin cell wall as a result of the action of the maceration enzymes. For the analysis the wine pH was adjusted to pH 3.5 and an excess of acetaldehyde were added to eliminate the bleaching effect of SO_2 after which the absorbance of samples was measured at OD_{420} and $OD_{520.}$ The sum of the measurements is the value for the colour density of the wine.

In a study[3] where the grape phenolics of Pinotage were compared with four other red cultivars (Merlot, Shiraz, Cabernet franc and Cabernet Sauvignon), Pinotage had the highest anthocyanin (mg/g berry) and total phenolics (mg/g berry) content of all five cultivars. When comparing the modified color densities of the wines from the different cultivars, Pinotage had the second highest value (11.65) after Shiraz (12.3), placing it in the "deep red" wine color category. The modified color density of the control wine of 2012 from this study compares well with the results from Du Toit[3] having a modified color density of 11.8, but in 2013 the value for the control wine was only 9.7. According to Fournand and others[4] some cultivars exhibit a decline in anthocyanin content near or after maturity and this can be ascribed to β-glycosidase and peroxidase activity.

For both the 2012 and 2013 vintages, treatment with maceration enzymes did not improve the color density of the resulting wines, with color densities only slightly higher, equal to, or even lower than the control fermentation. This emphasizes the value of the CoMPP method that shows the direct impact of the enzymes on the cell wall composition.

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SUPPLEMENTARY TABLES AND FIGURES

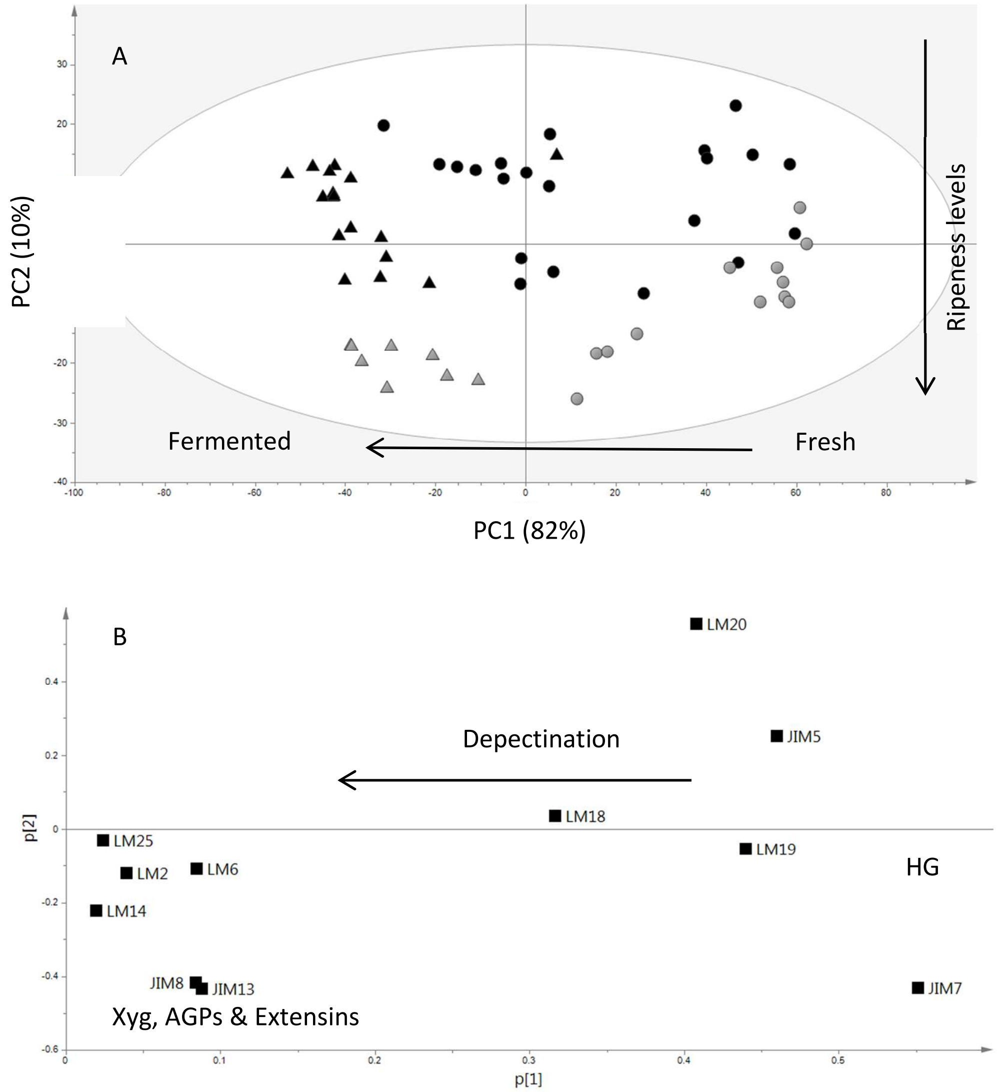
Table S1. Chemical properties of Pinotage juice (before yeast inoculation) and the resulting wine at the end of alcoholic fermentation for 2012 and 2013. Colour density (420 + 520 nm) was determined after three months of bottle aging. The values are the average of three biological repeats. Only the volatile acidity of 2012 showed a statistical significant difference between the values and they were marked with letters to indicate the differences (unpaired T-test, 95% confidence interval) between them. Fermented = wine without enzyme addition. ExCol, Expr and CB is wine fermented with the addition of Rapidase® Ex Color, Rapidase® Expression and Rapidase® CB respectively.

Figure S1. CoMPP results showing the effect that the fermentation has on the cell wall composition in the CDTA fraction;(A) score plot and (B) loading plot. Fresh grape skin cell walls (22.7 °B, 2012, ● black circle and 26.5°B, 2013, ● grey circle) are compared to fermented (without enzymes) (22.7 °B, 2012, ▲ black triangle and 26.5°B, 2013, ▲ grey triangle) cell walls. Xyg, xyloglucan.

Figure S2. The effect of maceration enzymes on the FT-MIR spectra of the skin cell walls. Similarly to Figure 7 there is an increase in cell wall proteins (exposure on surface of AIR particles) and a decrease in pectins for enzyme treated skins compared to fermented (without enzyme addition) skins. The spectra represent the average absorbance from at least five AIR samples per treatment. Control = Fermented cell wall samples without enzyme addition, ExCol, Expr and CB = Fermented cell wall samples with the addition of Rapidase®ExColor, -Expression and –CB respectively, 12 = 2012 and 13 = 2013.

Table S1.

		pH		Volatile acidity		Total Acid (g/L)		Gluc + Fruc (g/L)		Ethanol %		Colour density	
2012	Juice	3.28	±0.02			5.37	±0.11	227	±1.42				
	Fermented	3.39	±0.01	0.43[a]	±0.03	6.59	±0.09	1.30	±0.14	14.32	±0.12	11.76	± 1.09
	ExCol	3.35	±0.04	0.45[a]	±0.04	6.87	±0.29	1.29	±0.02	14.07	±0.38	12.39	± 0.65
	Expr	3.40	±0.02	0.50[b]	±0.01	6.60	±0.14	1.30	±0.11	14.15	±0.10	12.34	± 0.68
	CB	3.40	±0.01	0.47[a]	±0.03	6.62	±0.12	1.28	±0.06	14.38	±0.19	12.33	± 0.97
2013	Juice	3.71	±0.07			4.85	±0.20	265	±0.96				
	Fermented	3.71	±0.04	0.24	±0.04	4.47	±0.19	3.51	±0.60	14.55	±0.49	9.66	± 1.74
	ExCol	3.63	±0.07	0.25	±0.02	4.64	±0.15	3.12	±0.54	13.36	±1.60	8.78	± 1.71
	Expr	3.75	±0.04	0.32	±0.04	4.47	±0.16	3.71	±0.38	14.21	±0.48	9.61	± 0.71
	CB	3.74	±0.04	0.32	±0.03	4.44	±0.10	3.03	±0.68	13.93	±0.90	9.57	± 0.72



A
PC2 (10%)
PC1 (82%)
Fermented
Fresh
Ripeness levels
B
LM20
JIM5
Depectination
LM18
LM25
LM19
HG
LM2
LM6
LM14
JIM8
JIM13
JIM7
Xyg, AGPs & Extensins
p[1]
p[2]

Figure S1

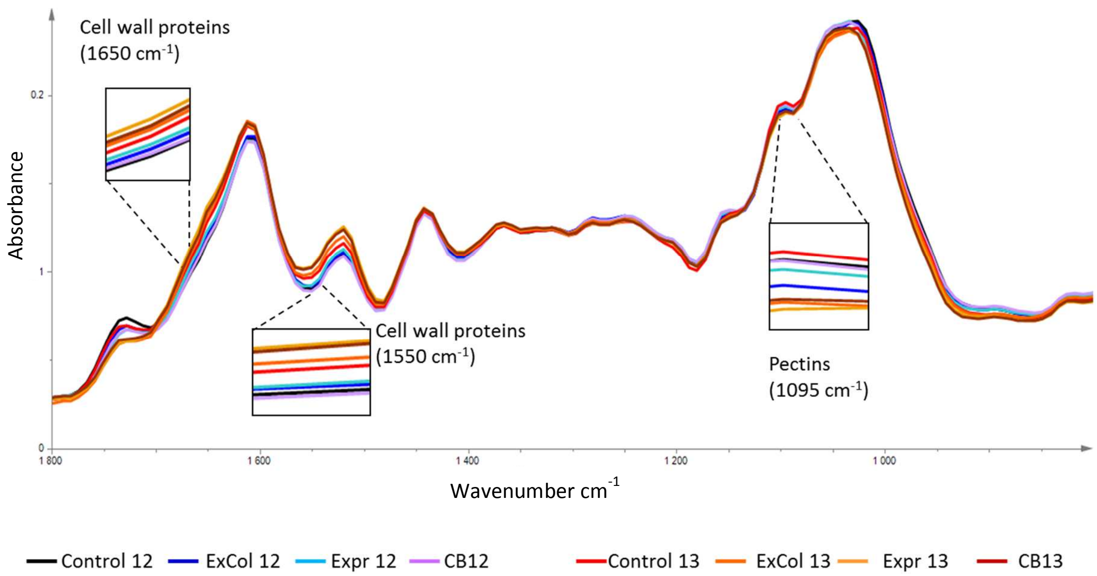

Cell wall proteins
(1650 cm-1)
Cell wall proteins
(1550 cm-1)
Pectins
(1095 cm-1)
Absorbance
0.2
1
0
1 800
1 600
1 400
1 200
1 000
Wavenumber cm-1
Control 12
ExCol 12
Expr 12
CB12
Control 13
ExCol 13
Expr 13
CB13

Figure S2.