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釀酒葡萄渣於葡萄酒精煉應用之探討

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摘要

葡萄渣作為葡萄酒釀製過程中產生的副產物，為葡萄酒業主要有機固體廢棄物。因此尋求增加其附加價值之方法。部分研究指出，釀酒葡萄渣對花青素原及丹寧具有較高親和性。因此本篇報告將探討葡萄渣於葡萄酒業之天然精煉劑應用。於實驗室規模下測試慕和懷特(MOpCWs)及卡本內蘇維農葡萄(CSpCWs)細胞壁萃取物對慕和懷特紅酒單寧及花青素濃度之變化，結果顯示 pCWs 對慕和懷特紅酒有較好的花青素(48%至68%) 及單寧吸附能力(44 至 66%)，其中以 MOpCWs 效果最好。於因子分析實驗中，發現於單寧缺乏的芳特娜雜合紅酒發酵前移除葡萄渣並外加 3g/L 單寧可提升 2.4 倍的單寧，其中 flavan-3-ols 提升至 27.8%。於實驗室規模下，葡萄籽粉末應用於四種白酒 Fiano (FN)、Sauvignon blanc (SAB1)、Semillon/Sauvignon blanc blend (SEM/SAB)、Marsanne (MR)及兩種果汁 Semillon (SEM)和 Sauvignon blanc (SAB2)的穩定性測試中顯示葡萄籽粉末具有穩定上述六種樣品的能力，且 GSP 添加於果汁中比酒中有更好的酒霧蛋白降低能力。由此觀察控制某些品種葡萄渣存在於紅酒中與單寧酸濃度變化之應用及穩定白酒品質上，作為非致過敏之天然物質，可能有作為精煉劑應用於非雜合品種葡萄酒之潛力。

一、 前言

葡萄作為世界重要經濟作物，年產量約 5 億噸 (FAOSTAT, 2014; Scoma *et al.*, 2014)，用於釀酒的葡萄佔約 75% (Zhu *et al.*, 2015)。而釀酒葡萄渣(wine grape pomace)為葡萄酒釀造產生的固形物，葡萄擠壓破碎後產生果汁(must)、果皮、種子及果梗。為葡萄酒釀造業中主要產生的有機固體廢棄物(Abarghuei *et al.*, 2010; Christ and Burrit, 2013; Cuccia, 2015; Beres *et al.,* 2017)。最近部分研究指出釀酒葡萄渣對花青素原有很高的親和性(Bindon *et al.*, 2013)。

精煉的目的為移除酒中可能帶來負面品質影響的組成分並感官品質。傳統上是為了減少酒中過多可能帶來苦澀或混濁的酚類(Jiménez-Martínez *et al.*, 2017)。一些蛋白質，例如:明膠(gelatin)、白蛋白(albumin)或酪蛋白(casein)一直有作為精煉劑使用(Sarni-Manchado *et al.*, 1999)。然而近年研究顯示部分動物性蛋白有造成過敏的風險，例如:牛明膠、酪蛋白和白蛋白，因此期望尋找可替代的植物蛋白精煉劑(Marchal *et al.*, 2002; Maury *et al.*, 2003)。

單寧(Tannins)作為紅酒中重要的酚類化合物分為:flavan-3-ol 寡聚物、聚合物，對紅酒顏色穩定性與口感有重要的影響(Moreno-Arribas & Polo, 2009; Ribéreau-Gayon, Glories, Maujean, & Dubordieu, 2006)。紅酒的澀味與單寧濃度、聚合度(mean degree of polymerization, mDP)及沒食子酸酯化(galloylation)呈正相關(Ma *et al.*, 2014)。雜合品種紅葡萄會賦予葡萄酒特殊草味、不穩定顏色、高酸度和低單寧濃度(低於 100mg/L catechin 當量)(Manns, Coquard Lenerz, & Mansfield, 2013; Pedneault, *et al.*, 2013; Sun, Gates, Lavin, Acree, & Sacks, 2011)。

本報告將探討葡萄渣於葡萄酒精煉之應用與葡萄酒中單寧濃度之變化。

二、釀酒葡萄細胞壁物質於紅酒精煉之應用及對紅酒酚類組成之影響

Jiménez-Martíneze *et al.* (2017)參考 De Vries *et al.* (1981)，並被 Apolinar-Valiente *et al.* (2010)修改的作法製備 CWM。經過標準或浸解酶釀製紅酒後， 慕和懷特(Monastrell, MO)與卡本內蘇維儂葡萄(Cabernet Sauvignon, CS, VIVC-1929)渣分離出。10g 葡萄皮經 15mL 沸水煮沸 5 分鐘後均質，用 96%乙醇於 40°C下萃取 30 分鐘。溶於酒精的固體(Alcohol-insoluble solids, AIS)以濾紙過濾後，再用 70%乙醇萃取至幾乎無醣類存在於 70%乙醇相。之後 AIS 用丙酮加 96%乙醇及 96%乙醇各沖洗一次，最後於 20°C抽風櫃烘乾。得到的 CWM 使用劑量為 13mg/mL(Apolinar-Valiente *et al.*, 2015)。CWM 與 2.5mL MO 紅酒以 300rpm 混合 90 分鐘再以 18000g 離心取上清液。分別以 Phloroglucinolysis (PG)法和膠體層析法進行單寧分析。以 HPLC 定量花青素(2.5×0.4cm, 5μm C-18 管柱，溶劑 A 和 B 分別為 formic acid 和 acetonitrile)。得到的目標物質以 malvidin-3-glucoside 為外標進行定量。MO CWM 及 CS CWM 組(依照標準及浸解釀製又分 p1 和 p2)的結果同時與使用商業精煉劑(依照建議最大與最小劑量分為 1 和 2)的組別:明膠(gelatin, G1 與 G2 (高水解度與中水解度))、豌豆蛋白(pea protein, PP)、酵母菌產物(yeast-derived compound, YM)進行比較。結果顯示(Table 1.)所有組別均會降低紅酒中花青素含量(保留 25 至 68%)，MOpCWs、CS p2CW 和 YM2 保留最多(64%)。pCWs 可能透過離子鍵與疏水性交互作用接上多醣表面，造成聚集而形成一層膜(Guerrero *et al.*, 2013)。此外，所有精煉劑均會降低單寧濃度(Table 2.)，其中以 MOpCWs、PP 保留最多(64%)，且由 mDP 數值顯示 MOpCWs 可能偏好保留低分子量單寧。然而所有組別使用 SEC 法呈現的單寧保留量大多低於 Phloroglucinolysis 法(Table 3.)，只有 CSpCWs 的數值於兩種測試方法中接近(但 pCWs 仍是所有組別中數值差異最小的)。可能是因為它們能夠抓住無法透過 PG 法分解的氧化單寧。

三、釀酒葡萄渣限制芳特娜葡萄酒單寧保留

變量包含:精煉(不加、bentonite、加熱)；和酒渣一起發酵(有、無)；外加單寧量(0、1、3、9g)；浸漬時長(0、4、11 天)共 72 組。芳特娜葡萄(red Frontenac, Landot (L. 4511) X Vitis riparia 89)擠壓破碎後，果汁與其葡萄渣以特定比例加入 25L 發酵槽中，加入 30 mg/L 的 SO_2 於 4℃下浸泡 4 天低溫預發酵，之後壓榨(1.8bar)成泥分裝，反應一段時間後再分裝用於移除蛋白質的精煉測試:添加 30g bentonite、水浴加熱 60 或 80℃ 20 分鐘。之後進行發酵則裝於 1L 玻璃罐中，將 500mL 有(無)添加精煉劑的果汁用於後續單寧及葡萄渣添加測試。使用 250mg/L 商業酵母 *Saccharomyces cerevisiae* 24℃酒精發酵後，進行 HPLC-FLD 分析。依上述變量條件繪製一張熱地圖(Figure 3.)，統計數據顯示(Table 4.) :芳特娜紅酒中蛋白質濃度及紅酒顏色會與精煉方式、添加單寧、葡萄渣與否或浸漬時長有很大關聯且幾種變量之間不互相獨立。控制組紅酒中蛋白質濃度於酒精發酵終止(end of alcoholic fermentation, eAF)前後下降 4.9 倍。於加熱組中添加 9g/L 單寧會比同樣條件下的控制組，蛋白質濃度要高(Table 5.)。這指出葡萄酒基質中可能有保留蛋白質的物質。此外，外加單寧也會使蛋白質沉澱。於只添加單寧的控制酒中發現對雜合紅酒於 eAF 及其後第 11 天之蛋白質濃度影響不大。然而未加葡萄渣，添加單寧的控制紅酒在 eAF 時，蛋白質濃度降低控制組的 2.8 倍，並於 eAF 後第 11 天，降低至 2.9 倍；添加葡萄渣，且外加單寧，且使用 bentonite 的紅酒中，蛋白質濃度會上升。然而在 eAF 後第 11 天，蛋白質濃度會低於用 bentonite 或加熱的含葡萄渣紅酒。這項結果顯示葡萄渣可能是蛋白質存留於芳特娜紅酒中的主要原因。有加熱的含葡萄渣紅酒對總色素含量(totalment, Tpg)或花青素單體皆不影響，但會減少紅酒中的聚合花青素，這顯示單體花青素於酒精發酵前達到頂峰(Figure. 4)。可能低溫預發酵及加熱造成細胞壁破壞釋放色素。若為 bentonite 加葡萄渣之紅酒，可以改善聚合花青素的形成及單體花青素的萃取/保留。去除蛋白質、添加葡萄渣或單寧、浸漬時長對芳特娜中單寧濃度之影響(Figure 5.)，去除蛋白質對單寧濃度影響不大，但其他變量之間交互作用影響很大($p<0.05$)。葡萄渣存在會大幅影響該紅酒中單寧的萃取/保留。沒加葡萄渣且加 3 與 9g/L 單寧的紅酒比使用葡萄渣的組別，單寧濃度高很

多，又為控制組單寧含量的 2.4 倍。主要為 oligomeric flavan-3-ols，polymeric flavan-3-ols。蛋白質限制單寧保留，葡萄渣傾向增加紅酒中蛋白質與色素分子，因此葡萄渣限制單寧保留(Bautista-Ortín *et al.*, 2016; Kilmister *et al.*, 2014)。添加 3g/L 以上單寧可大幅增加單寧濃度，沒有葡萄渣的情況下更好，但在 eAF 後第 11 天，單寧濃度快速下降且 WOP 組比 WP 組降更多(1.5 至 2.5 倍)。可能來自花青素與花青素原聚合(Cheynier *et al.*, 1998)，或外加單寧與紅酒本身單寧產生聚合(Gambuti *et al.*, 2010)。

四、初次測試葡萄種子粉末替代膨潤土之可行性

葡萄籽分離自未發酵之冷凍夏多內葡萄並於 180℃烘烤 10 分鐘後直接碾碎成粉，並與馬克葡萄的籽粉比較。此外，亦對未烘烤葡萄籽進行比較。在酒中分別添加 5g 至 35g；1g 至 5g 葡萄籽粉末(grape seed powder, GSP)，並接觸 1 小時或 2 小時以評估四種白酒: Fiano (FN)、Sauvignon blanc (SAB1)、Semillon/Sauvignon blanc blend (SEM/SAB)、Marsanne (MR)之熱穩定性，控制組為 bentonite。兩種果汁: Semillon (SEM)和 Sauvignon blanc (SAB2)中則分別加 5、10、20g/L GSP，20℃下接觸 1 小時，控制組未添加 GSP。之後對樣品中組成和顏色進行測定(Table 6.) :用 infered 測 pH 與殘留醣、UHPLC 測定會使酒壞掉的蛋白質(Grape pathogenesis-related, PR protein)包含 Thaumatin Like Proteins (TLPs)和 chitinases，之濃度與組成、用 UV-Vis 於 OD280 測酚類的 catechin 當量、用 CIELAB 測定酒顏色。結果顯示 GSP 反應 1 小時後會使不穩定白酒中的蛋白質濃度下降(Figure 1.)，相較控制組 FN 、SAB1、SEM/SAB、MR，在 5g/L GSP 加入反應 1 小時後 PR 蛋白濃度分別下降 53%、45%、62%和 48%且隨 GSP 添加劑量上升而上升。SAB1 與 MR 於更高劑量時，PR 蛋白移除的比率接近。但對 SEM/SAB 移除 TLP 的效果不太好。GSP 亦可減少酒霧(haze)形成(Figure 6.)。FN 的酒霧隨 GSP 添加量上升而下降，SAB1、SEM/SAB、MR 分別在 5 或 10g/L GSP 時，酒霧增加，直至 15g/L 添加量才下降。這可能是因為酚類化合物量不足，無法全部接上的酒霧中的蛋白質，進而無法移除

酒中的蛋白質。GSP 富含多酚類，因此 OD280 會上升(Ribéreau-Gayon *et al.*, 2000)。這也解釋了 GSP 會釋出多酚至白酒中，與 PR 蛋白接觸的假設(Siebert, 2006)。殘糖可能覆於 GSP 表面，改變酒的味道造成不好影響。此外，GSP 添加會讓酒的彩度下降使顏色變暗，降低顧客喜愛，且相較控制的 bentonite 組，澄清度會下降。除了 MR 無顯著差異(偏黃)外，其他會偏綠。於 SEM 加入 5g/L GSP，分別可降低 85%酒霧蛋白，並移除 chitinases 及 75%TLP；同樣劑量 SAB2 果汁中會降低 71%酒霧蛋白，且 chitinases 及 TLP 均會下降。這個結果顯示 GSP 作為澄清劑加入果汁中比加入酒中，去除酒霧蛋白的效果還好。可能是因為沒有乙醇存在的關係(Singleton & Draper, 1964) 。但添加 GSP 於果汁中對顏色的影響比添加於酒中要少得多，且對果汁組成的影響比添加 bentonite 少。

五、 總結

在實驗室規模下萃取 MOpCWs 和 CSpCWs 並應用於慕和懷特紅酒精煉，同時與常用動物性蛋白精煉劑進行比較。結果顯示 pCWs 對慕和懷特紅酒具有良好的花青素及單寧吸附能力，這可能能夠改善感官上的苦味及澀味。其中以 MOpCWs 和 PP 降低單寧的效果最佳。在因子分析的測試中，葡萄渣被發現並不能作為天然單寧的提供者，相反的會限制單寧缺少的雜合紅酒芳特娜保留單寧的能力。於芳特娜紅酒發酵中，發酵前移除葡萄渣，並外加 3g/L 單寧，可以大幅增加芳特娜紅酒中的單寧。在實驗室規模下測試白葡萄籽 GSP 添加於四種白酒及兩種果汁中，結果顯示 GSP 可以移除酒霧蛋白，於果汁中添加比在酒中添加有更好的酒霧蛋白移除能力，且對組成、發酵時間不會影響。由此觀察特定品種葡萄渣於紅酒類單寧酸濃度應用及穩定白酒品質上，作為非致過敏之天然物質，可能有作為精煉劑應用於葡萄酒工業之潛力。

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七、 圖表

7.1 釀酒葡萄細胞壁物質於紅酒精煉之應用及對紅酒酚類組成之影響

Table 1. Concentration of the remaining anthocyanins in wine before and after the use of different fining agents.

	Del-Glu	Cyan-Glu	Pet-Glu	Peon-Glu	Malv-Glu	AC	AT	% adsorption*
Wine	49.4 ± 2.9 h	14.2 ± 0.7e	75.2 ± 4.7f	41.6 ± 3.3 g	322.4 ± 21.8e	89.8 ± 0.9 g	592.6 ± 32.6f	
Wine + G1.1	36.5 ± 3.4f	9.7 ± 0.9d	55.1 ± 5.9de	31.2 ± 3.8f	241.5 ± 26.9d	68.7 ± 6.9f	442.7 ± 47.7e	25.3a
Wine + G1.2	36.5 ± 4.2f	9.9 ± 0.9d	55.4 ± 6.4e	30.4 ± 3.0f	237.1 ± 28.2d	67.4 ± 7.1ef	436.7 ± 49.7e	26.3a
Wine + G2.1	29.1 ± 1.0cde	7.8 ± 0.3bcd	43.5 ± 2.1c	25.2 ± 1.6 cdef	196.0 ± 12.6cd	56.5 ± 2.0de	358.1 ± 19.6cd	39.6bc
Wine + G2.2	25.8 ± 2.1cd	7.5 ± 0.5bc	39.0 ± 2.4bc	22 ± .01.7bcde	171.4 ± 15.4bc	48.7 ± 4.6cd	314.4 ± 26.7c	46.9bc
Wine + PP 1	31.7 ± 4.9ef	8.9 ± 1.4cd	46.9 ± 7.0cde	26.9 ± 4.4ef	206.4 ± 28.8cd	59.3 ± 8.5fg	380.1 ± 55.0de	35.9ab
Wine + PP 2	30.6 ± 0.8de	8.6 ± 0.2cd	45.4 ± 1.2cd	25.7 ± 0.4def	200.4 ± 10.4cd	57.1 ± 2.8ef	367.9 ± 15.8cd	37.9bc
Wine + YM 1	20.3 ± 4.4ab	6.1 ± 1.1ab	30.4 ± 5.8ab	17.8 ± 3.4abc	129.9 ± 23.4ab	40.4 ± 7.0bc	245.0 ± 44.9ab	58.6de
Wine + YM 2	17.2 ± 1.8a	5.3 ± 0.4a	25.5 ± 2.4a	14.4 ± 1.4ab	109.1 ± 9.0a	34.1 ± 3.8ab	205.6 ± 18.6a	65.3e
Wine + MO p1CW	16.1 ± 1.7a	5.2 ± 0.6a	24.3 ± 3.0a	14.2 ± 2.3a	101.1 ± 14.6a	25.9 ± 2.5a	186.9 ± 24.5a	68.5e
Wine + MO p2CW	19.7 ± 0.6ab	5.9 ± 0.2ab	30.3 ± 0.1ab	18.6 ± 0.1abcd	130.1 ± 0.0ab	30.7 ± 1.2a	235.2 ± 2.2a	60.3e
Wine + CS p1CW	24.8 ± 3.0bc	7.4 ± 0.9bc	38.9 ± 4.0bc	23.8 ± 2.5cdef	172.4 ± 17.4bc	39.4 ± 3.7b	306.8 ± 30.9bc	48.2cd
Wine + CS p2CW	24.1 ± 5.1bc	4.6 ± 3.7a	25.1 ± 15.1a	28.8 ± 13.6ef	108.9 ± 74.1a	40.3 ± 5.8b	231.9 ± 74.1a	60.9e

Abbreviations: Calculated as the difference between anthocyanin concentration in the solution before and after use of different fining agents. Del-Glu delphinidin-3-glucoside, Cyan-Glu cyanidin-3-glucoside, Pet-Glu petunidin-3-glucoside, Peon-Glu peonidin-3-glucoside, Malv-Glu malvidin-3-glucoside, AC sum of acylated anthocyanins, AT total anthocyanins, % adsorption was calculated on total anthocyanin content, G1 gelatine-Vinigel Seda used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, G2 gelatine-Vinigel Cristal used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, PP vegetal protein-Proveget 100 used at the minimum (1, 0.05 g/L) and maximum (2, 0.2 g/L), YM yeast autolysate used at the minimum (1, 0.2 g/L) and maximum (2, 0.5 g/L) dose, MO/CS PCW cell wall isolated from Monastrell/Cabernet Sauvignon pomace of a standard red wine vinification (1) and a red wine vinification where a maceration enzyme was applied

(2). Different letters within same column indicate significant differences ($p < 0.05$) according to a LSD test.

(Jiménez-Martínez *et al.*, 2017)

Table 2. Concentration and composition of the remaining proanthocyanidins in wine before and after the use of different fining agents.

	TT (mg/L)	% adsorption*	mDP	EGC (μM)	ECG (μM)
Wine	1043.3 ± 57.1h		6.1 ± 0.1b	1811.8 ± 57.2h	227.3 ± 11.9h
Wine + G 1.1	732.6 ± 6.5g	29.8a	6.1 ± 0.1b	1273.1 ± 12.1g	158.8 ± 1.9g
Wine + G 1.2	676.2 ± 37.4f	35.2b	6.5 ± 0.0c	1181.5 ± 21.8f	149.8 ± 9.6fg
Wine + G 2.1	580.9 ± 33.2e	44.3c	6.1 ± 0.2b	992.5 ± 11.9e	106.8 ± 6.2d
Wine + G 2.2	517.1 ± 6.0d	50.4d	5.9 ± 0.2b	895.0 ± 9.6d	94.1 ± 1.7c
Wine + PP 1	439.3 ± 19.4c	57.9e	6.8 ± 0.2d	790.5 ± 17.1c	95.5 ± 1.5cd
Wine + PP 2	371.5 ± 6.4ab	64.4f	6.6 ± 0.1cd	773.1 ± 28.0c	76.0 ± 1.8b
Wine + YM 1	370.3 ± 5.6ab	64.5f	6.9 ± 0.3d	598.7 ± 20.2ab	68.1 ± 1.8ab
Wine + YM 2	343.4 ± 12.1a	67.1f	6.5 ± 0.3c	544.3 ± 17.2a	64.0 ± 3.1a
Wine + MO p1CW	376.6 ± 7.9ab	63.9f	5.9 ± 0.1b	607.1 ± 19.7ab	91.0 ± 0.2c
Wine + MO p2CW	410.2 ± 12.4bc	60.7e	5.6 ± 0.1a	625.8 ± 17.5b	99.3 ± 4.0cd
Wine + CS p1CW	552.4 ± 34.8de	47.1c	6.6 ± 0.1cd	944.9 ± 17.8de	131.2 ± 5.4e
Wine + CS p2CW	585.5 ± 11.3e	43.9c	6.7 ± 0.0cd	1002.2 ± 33.8e	144.7 ± 6.8f

Abbreviations: * Calculated as the difference between PA concentration in wine before and after use of different fining agents. TT total tannins, EGC (−)-epigalocatechin, ECG (−)-epicatechingalate, G1 gelatine-Vinigel Seda used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, G2 gelatine-Vinigel Cristal used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, PP vegetal protein-Proveget 100 used at the minimum (1, 0.05 g/L) and maximum (2, 0.2 g/L), YM yeast autolysate used at the minimum (1, 0.2 g/L) and maximum (2, 0.5 g/L) dose, MO/CS pcw cell wall isolated from Monastrell/Cabernet Sauvignon pomace of a standard red wine vinification (1) and a red wine vinification where a

maceration enzyme was applied (2). Different letters within same column indicate significant differences ($p < 0.05$) according to a LSD test.

(Jiménez-Martínez *et al.*, 2017)

Table 3. SEC Percentage of tannin retention calculated in phloroglucinolysis (*) and of phenolic compounds measured by size exclusion chromatography analysis (**).

% tannin retention (*)		% phenolic retention (**)	% retention at different time ranges (**)		
			Time (min)		
			10–11.5	11.5–13	13.17
Wine + G 1.1	29	12.0	4.3	12.3	13.6
Wine + G 1.2	35	32.0	30.0	32.3	33.0
Wine + G 2.1	40	33.8	34.3	35.3	36.4
Wine + G 2.2	50	37.9	34.6	37.9	39.1
Wine + PP 1	57	35.6	27.0	33.3	36.0
Wine + PP 2	64	38.8	31.0	37.1	41.3
Wine + YM 1	64	32.6	14.2	31.5	36.0
Wine + YM 2	67	37.9	16.6	36.5	41.3
Wine + MO p1CW	60	60.6	55.0	53.7	63.9
Wine + MO p2CW	63	53.4	44.8	49.1	60.5
Wine + CS p1CW	43	50.8	50.1	46.8	55.4
Wine + CS p2CW	47	50.8	48.3	47.1	55.2

Abbreviations: G1 gelatine-Vinigel Seda used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, G2 gelatine-Vinigel Cristal used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, PP vegetal protein-Proveget 100 used at the minimum (1, 0.05 g/L) and maximum (2, 0.2 g/L), YM yeast autolysate used at the minimum (1, 0.2 g/L) and maximum (2, 0.5 g/L) dose, MO/CS pcw cell wall isolated from Monastrell/ Cabernet

Sauvignon pomace of a standard red wine vinification (1) and a red wine vinification where a maceration enzyme was applied (2)

(Jiménez-Martínez *et al.*, 2017)

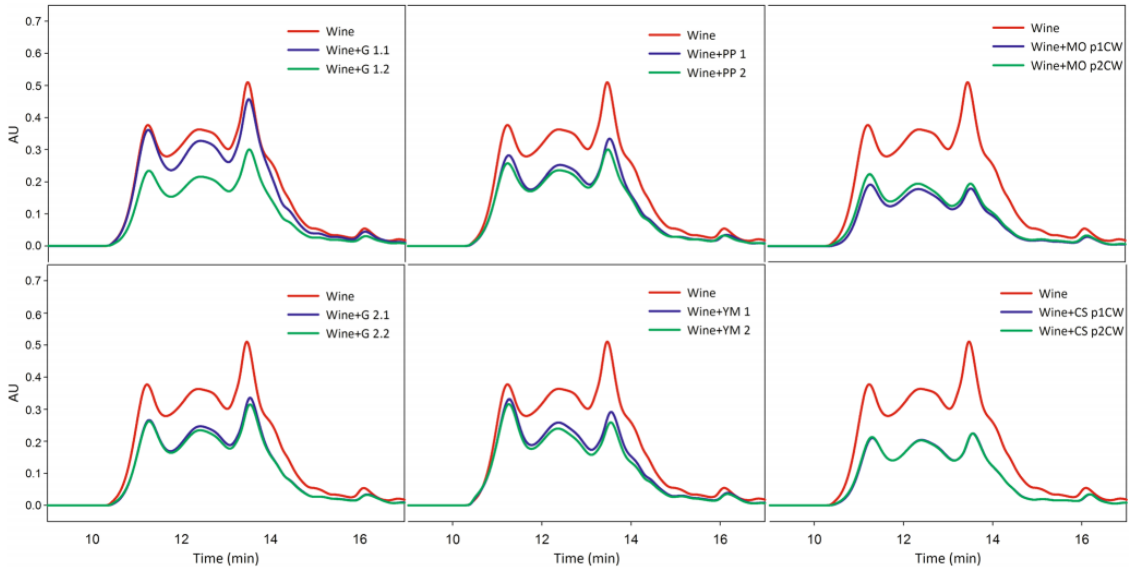


Figure 1. Comparison of the size exclusion chromatogram of the original proanthocyanidin in wine before and after the use of different fining agents. Abbreviations: G1 gelatine-Vinigel Seda used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, G2 gelatine-Vinigel Cristal used at the minimum (1, 0.3 mL/L) and maximum (2, 0.8 mL/L) dose, PP vegetal protein-Proveget 100 used at the minimum (1, 0.05 g/L) and maximum (2, 0.2 g/L), YM yeast autolysate used at the minimum (1, 0.2 g/L) and maximum (2, 0.5 g/L) dose, MO/CS pcw cell wall isolated from Monastrell/Cabernet Sauvignon pomace of a standard red wine vinification (1) and a red wine vinification where a maceration enzyme was applied (2).

(Jiménez-Martínez *et al.*, 2017)

7.2 釀酒葡萄渣限制芳特娜葡萄酒單寧保留

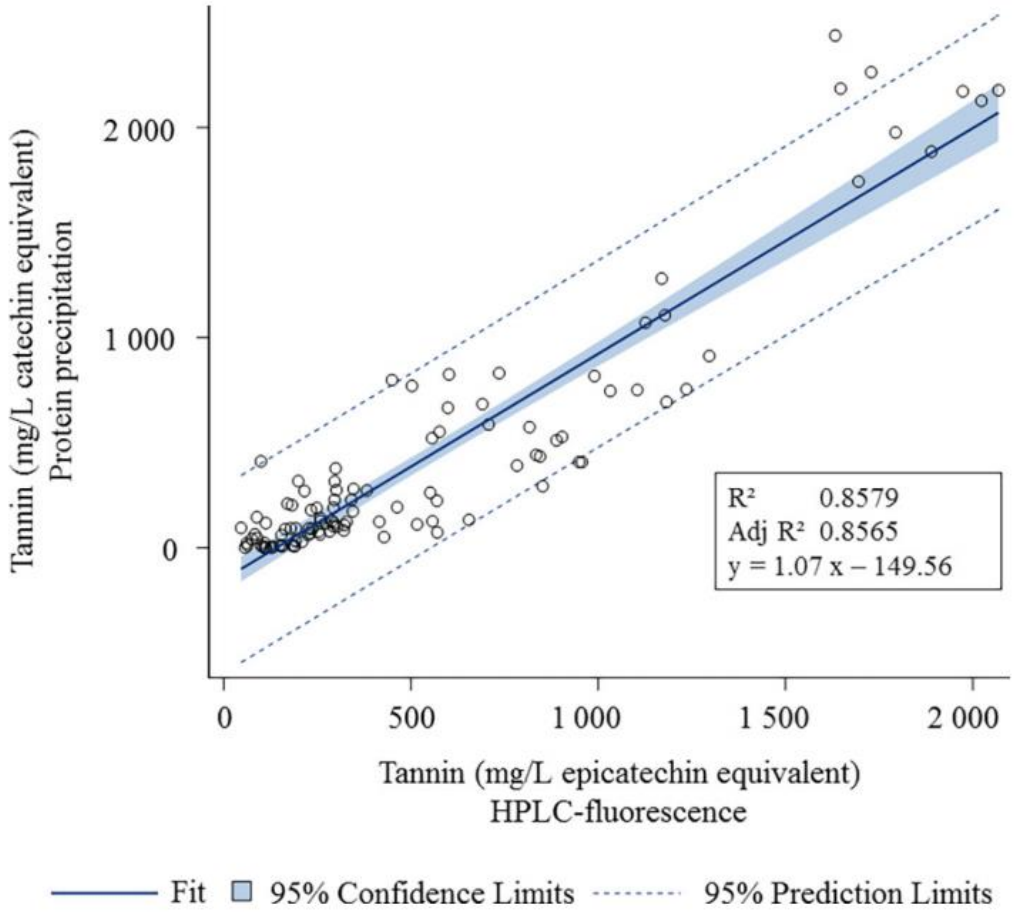


Figure 2. Regression of tannin concentration of experimental Frontenac wines as measured by protein precipitation and HPLC-fluorescence. Wine samples from the end of alcoholic fermentation (e-AF) and day 11 after the e-AF (corresponding to days 0 and 15 of the winemaking process, respectively) were included in the analysis.

(Paméla *et al.*, 2018)

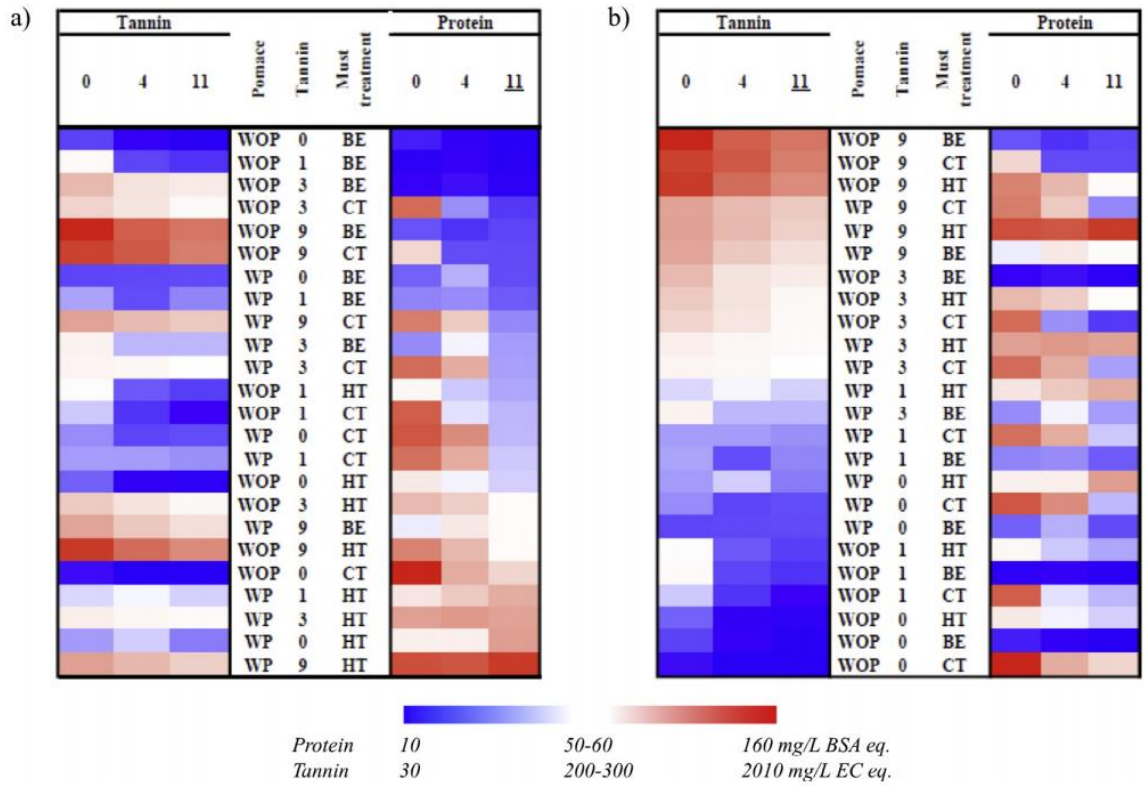


Figure 3. Heat map of the protein and tannin concentration of experimental Frontenac wines made with untreated (control, CT), bentonite-treated (BE), and heattreated (HT) must, fermented with (WP) and without (WOP) pomace, and with different doses of tannin addition (0, 1, 3, and 9 g/L) at 0, 4, and 11 days after the end of alcoholic fermentation (e-AF). Red and blue colours represent the highest and the smallest concentration of proteins or tannins in wines. Data are ranked from the lowest to the highest protein concentration, 11 days after the e-AF (a), and from the highest to lowest tannin concentration, 11 days after the e-AF (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Paméla *et al.*, 2018)

Table 4. Repeated measures analysis of variance (ANOVA) for protein, tannin, total pigment (Tpg), and co-pigmented, monomeric, and polymeric anthocyanin (CA, MA, and PP, respectively). Main effects are: must protein treatment (MT; untreated, bentonite-treated, and heat-treated must); pomace (P; must fermented with and without pomace); tannin addition (TA; 0, 1, 3, and 9 g/L); and time of maceration (TM; 0, 4, and 11 days after the end of alcoholic fermentation).

Effect	Protein		Tannin		Tpg		CA		MA		PP	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
MT	280.32	< 0.0001	2.48	0.1032	117.69	0.0003	134.05	< 0.0001	236.81	< 0.0001	156.33	< 0.0001
P	231.91	< 0.0001	1 411.91	< 0.0001	18.13	0.0003	9.01	0.0062	38.96	< 0.0001	6.82	0.0124
TA	17.13	< 0.0001	4 556.99	< 0.0001	2.95	0.0604	1.19	0.3426	13.48	< 0.0001	201.91	< 0.0001
TM	142.43	< 0.0001	659.07	< 0.0001								
MT*TM	104.29	< 0.0001	15.37	< 0.0001								
MT*P	13.91	< 0.0001	51.15	< 0.0001	42.05	< 0.0001	15.55	< 0.0001	20.40	< 0.0001	70.71	< 0.0001
MT*TA	56.84	< 0.0001	3.56	0.0102	7.07	0.0005	1.96	0.1258	1.05	0.4290	4.08	0.0026
P*TM	20.93	< 0.0001	125.07	< 0.0001								
TA*P	20.34	< 0.0001	1 152.41	< 0.0001	1.38	0.2723	1.68	0.1971	1.35	0.2821	54.17	< 0.0001
TA*TM	0.93	0.4798	185.55	< 0.0001								
MT*P*TM	21.42	< 0.0001	5.41	0.0007								
MT*TA*TM	3.24	0.0008	4.19	< 0.0001								
MT*TA*P	3.47	0.0071	7.64	< 0.0001	8.21	< 0.0001	5.14	0.0016	0.43	0.8514	2.32	0.0505
TA*P*TM	0.32	0.9265	16.51	< 0.0001								
MT*TA*P*TM	1.57	0.1194	3.09	0.0012								

(Paméla *et al.*, 2018)

Table 5. Protein concentration (mean ± standard deviation (SD), mg/L BSA equivalent) in experimental Frontenac wines made with untreated (control), bentonite-treated, and heat-treated must, fermented with (WP) or without (WOP) pomace, and with different doses of tannin addition (0, 1, 3, and 9 g/L) at the end of alcoholic fermentation (e-AF) and on the 4th and the 11th day following the e-AF (corresponding to days 4, 8, and 15 of the winemaking process, respectively).

Factor			Day after the e-AF (Time)		
			e-AF (day 0)	4	11
Pomace	Tannin (g/L)	Must treatment	Mean ± SD	Mean ± SD	Mean ± SD
WP	0	Control	129.43 ± 7.98 a A x *[a]	101.79 ± 10.35 B a x	42.70 ± 6.90 a C y *
		Bentonite	30.08 ± 3.50 b AB z	41.75 ± 7.95 b A y *	26.83 ± 4.22 b B z *
		Heat	58.50 ± 3.78 c B y	58.70 ± 11.58 d B x	93.64 ± 1.22 b A x *
	1	Control	115.59 ± 3.14 ab A x	87.52 ± 9.38 ab B x *	45.03 ± 1.98 a C y
		Bentonite	35.36 ± 4.69 ab A y *	36.54 ± 6.37 b A z *	29.14 ± 1.83 b A z *
		Heat	62.88 ± 5.17 c B z	74.66 ± 12.23 c AB x *	87.43 ± 17.39 b A x *
	3	Control	117.38 ± 6.55 ab A x	87.17 ± 8.11 ab B x *	39.33 ± 4.24 a C y *
		Bentonite	36.30 ± 6.80 ab A z *	50.70 ± 5.35 ab A y *	38.69 ± 4.54 b A y *
		Heat	92.59 ± 1.55 b A y	96.37 ± 10.94 b A x *	91.87 ± 9.85 b A x *
	9	Control	109.19 ± 12.44 b A x *	73.86 ± 5.91 b B y *	35.84 ± 1.97 a C z
		Bentonite	49.86 ± 10.35 a A z*	61.20 ± 5.77 a A y *	53.89 ± 11.50 a A y *
		Heat	133.55 ± 7.64 a A y *	129.16 ± 43.50 a A x *	145.78 ± 23.73 a A x *
WOP	0	Control	161.11 ± 15.75 a A x	87.35 ± 11.43 a B x	69.45 ± 9.18 a C y
		Bentonite	20.10 ± 7.51 a A z	15.04 ± 3.44 a A y	9.55 ± 0.67 b A z
		Heat	61.27 ± 8.89 c A y	50.84 ± 3.44 b A x	45.91 ± 21.18 a A x
	1	Control	125.73 ± 10.20 b A x	48.31 ± 3.11 b B x	42.53 ± 4.21 b B x
		Bentonite	12.82 ± 1.85 a A z	15.26 ± 2.51 a A y	10.61 ± 1.77 b A y
		Heat	55.45 ± 6.50 c A y	45.16 ± 4.76 b A x	40.24 ± 5.48 a A x
	3	Control	117.77 ± 36.49 b A x	36.88 ± 4.04 bc B y	24.11 ± 5.45 c B y
		Bentonite	16.26 ± 3.72 a A z	18.48 ± 0.57 a A z	13.42 ± 4.19 ab A z
		Heat	81.60 ± 20.38 b A y	72.81 ± 9.71 a A x	53.60 ± 7.37 a B x
	9	Control	69.06 ± 0.47 c A y	26.84 ± 5.28 c B y	26.64 ± 7.04 c B y
		Bentonite	27.83 ± 1.81 a A z	22.84 ± 2.88 a B y	25.92 ± 4.43 a C y
		Heat	106.17 ± 21.29 a A x	82.20 ± 7.95 a B x	53.95 ± 13.18 a C x

(Paméla *et al.*, 2018)

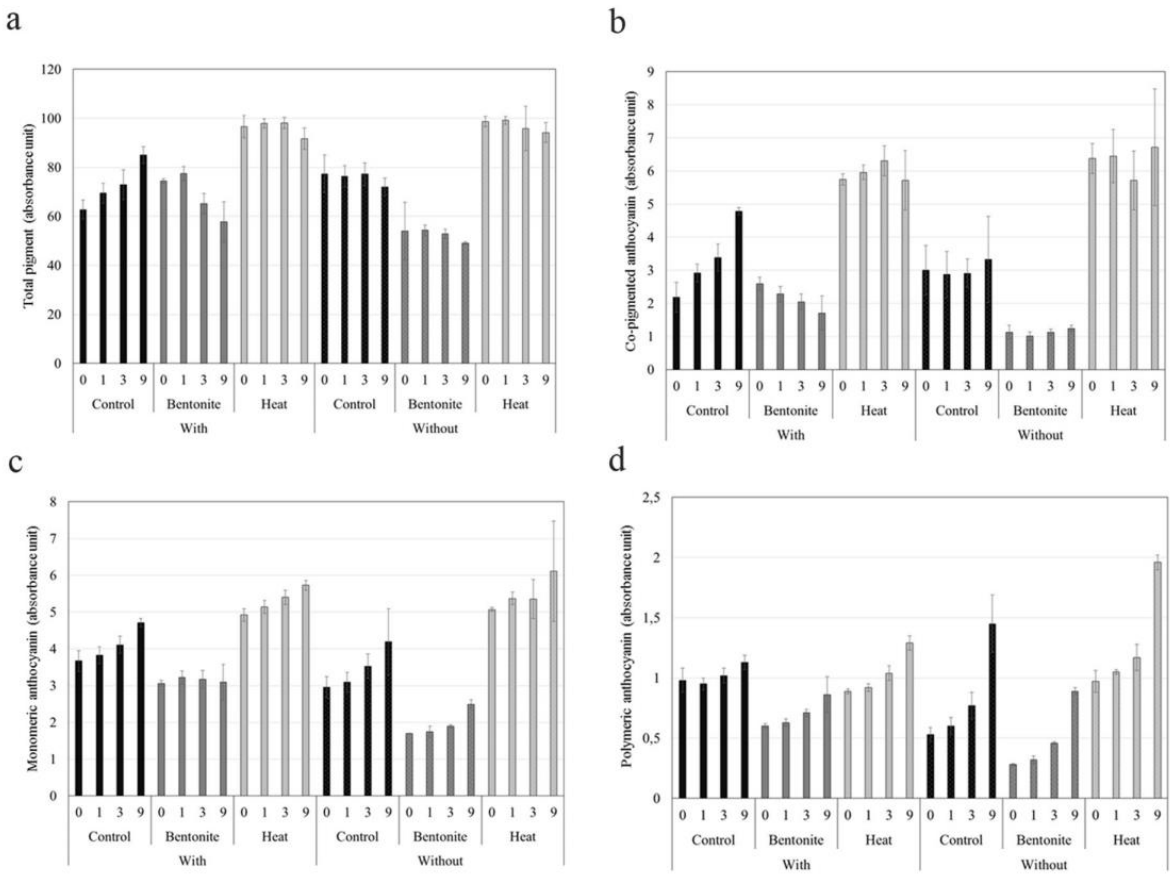
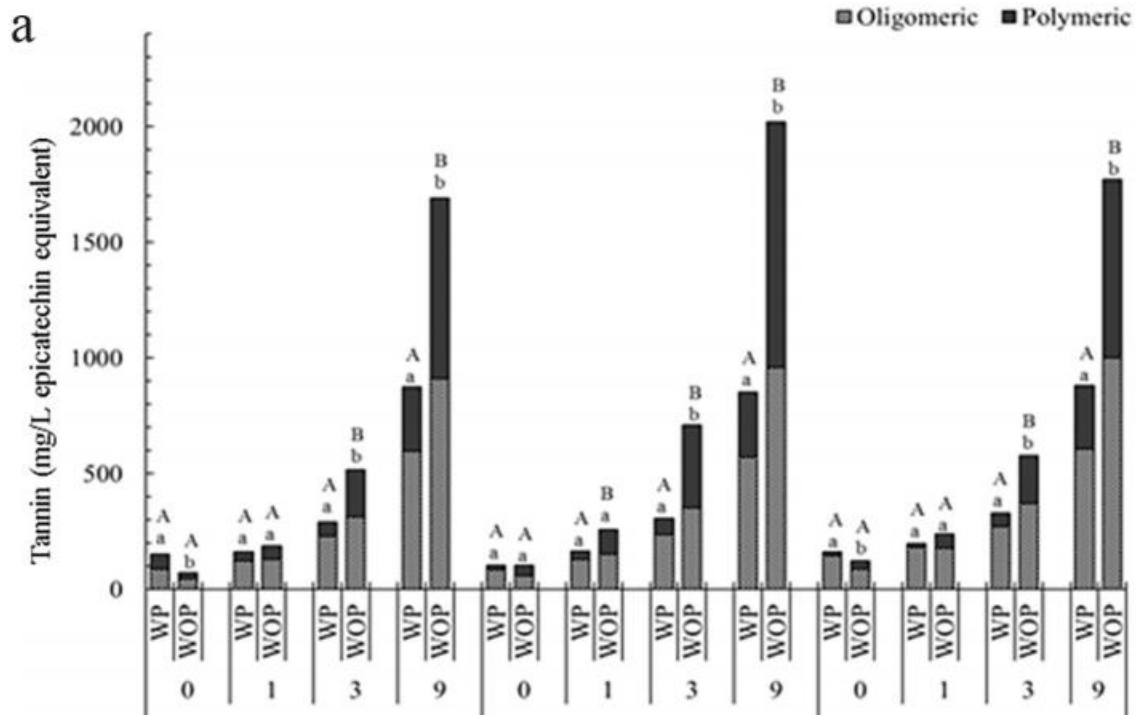
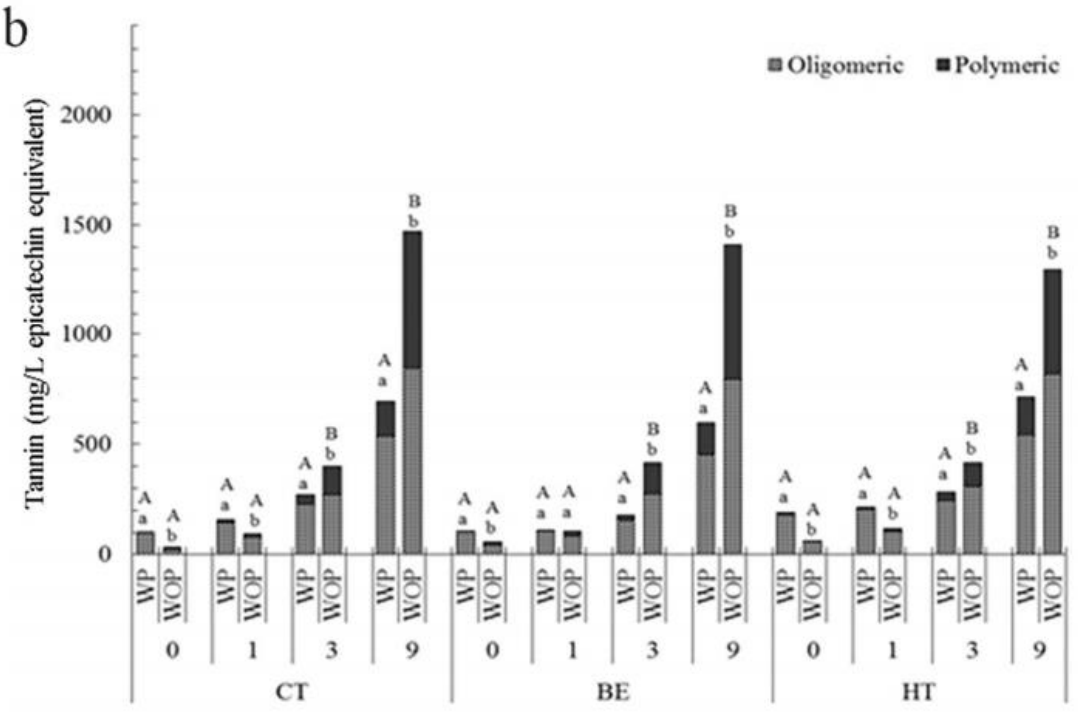


Figure 4. Total pigment (a) and co-pigmented (b), monomeric (c), and polymeric anthocyanin (d) estimation (in absorbance unit), in experimental Frontenac wines made with untreated (control), bentonite-treated, and heat-treated must, fermented with and without pomace, and with different doses of tannin addition (0, 1, 3, and 9 g/L) at 11 days after the end of alcoholic fermentation.

(Paméla *et al.*, 2018)


a
Oligomeric
Polymeric
Tannin (mg/L epicatechin equivalent)
2000
1500
1000
500
0
WP
WOP
0
1
3
9

1


b
Oligomeric
Polymeric
Tannin (mg/L epicatechin equivalent)
2000
1500
1000
500
0
WP
WOP
0
1
3
9
CT
BE
HT

2

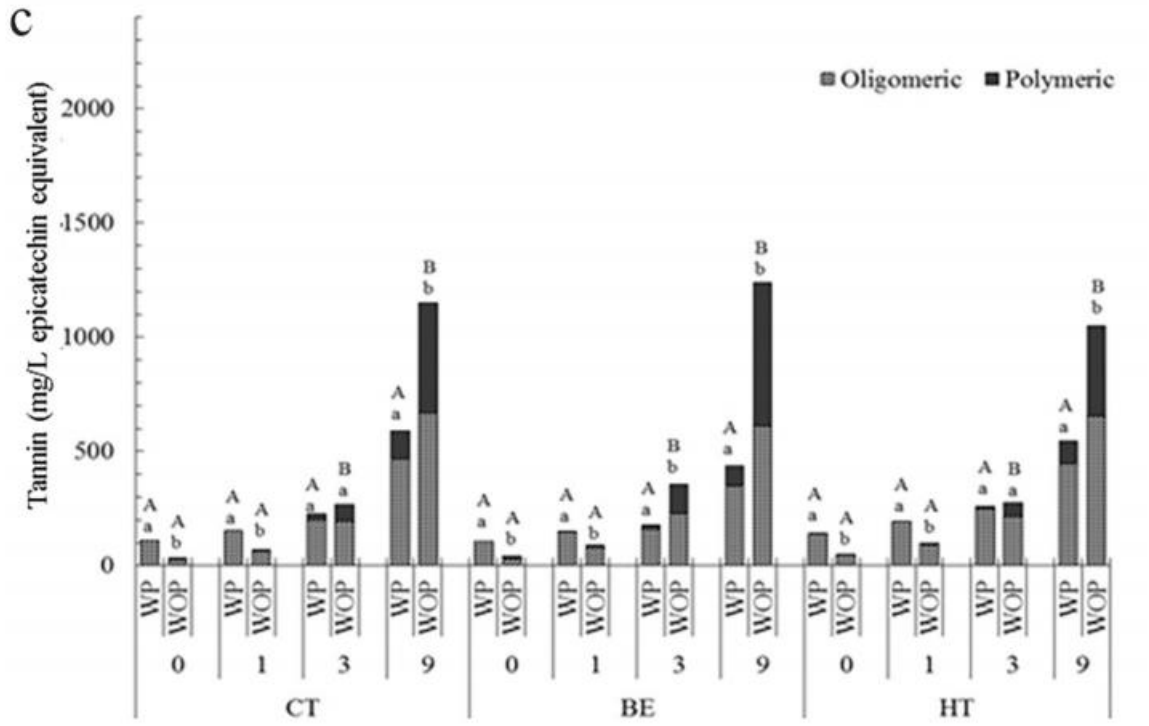


Figure 5. Oligomeric (2–5 units of flavan-3-ols) and polymeric (> 5 units of flavan-3-ols) flavan-3-ol concentration (mean, mg/L epicatechin equivalent) in experimental Frontenac wines made with untreated (control, CT), bentonite-treated (BE), and heat-treated (HT) must, fermented with (WP) and without (WOP) pomace, and with different doses of tannin addition (0, 1, 3, and 9 g/L) at the end of alcoholic fermentation (e-AF; a) and on the 4th (b) and the 11th (c) day following the e-AF (corresponding to days 4, 8, and 15 of the winemaking process, respectively). For a given combination of Dose*Treatment*Day, small letters compare the oligomeric flavan-3-ol concentration and capital letters compare the polymeric flavan-3-ol concentration of pomace treatment at the 0.05 probability level. The statistics for the other combinations are available in Supplementary Material.

(Paméla *et al.*, 2018)

7.3 初次測試葡萄種子粉末替代膨潤土之可行性

Table 6. Composition of selected wines with and without GSP (roasted and 1 h contact time) addition or bentonite treatment at the dose required to heat-stabilize each wine, including Fiano (FN), Sauvignon Blanc (SAB1), Semillon/SAB (SEM/SAB), Marsanne (MR). Results are shown as means and standard deviation of triplicate experiments. Sugars are expressed in g/L of glucose + fructose and the values of A280 are expressed in g/L of catechin equivalent. Different letters in the same column indicate statistically significant ($p < 0.05$) differences for each wine type.

Wines	Treatment	A_{280}	pH	Sugars	ΔE	L	a	b	C	H
FN	Control	0.64 ± 0.05b	3.22 ± 0.02b	0.7 ± 0.0b	–	98.0 ± 0.0b	−1.14 ± 0.01b	7.40 ± 0.01b	7.49 ± 0.01b	98.78 ± 0.02a
	GSP 26 g/L	2.33 ± 0.14a	3.32 ± 0.01a	3.1 ± 0.1a	11.01 ± 0.45	94.7 ± 0.3c	−1.45 ± 0.09a	17.88 ± 0.36a	17.94 ± 0.36a	94.64 ± 0.35b
	Bentonite 0.6 g/L	0.55 ± 0.01b	3.21 ± 0.02b	0.7 ± 0.1b	2.79 ± 0.26	98.4 ± 0.1a	−0.38 ± 0.02c	4.73 ± 0.25c	4.75 ± 0.26c	94.56 ± 0.01b
SAB1	Control	0.56 ± 0.04b	3.17 ± 0.01b	0.5 ± 0.0b	–	99.6 ± 0.0a	−1.89 ± 0.02b	5.46 ± 0.00b	5.68 ± 0.16b	109.0 ± 0.2a
	GSP 28 g/L	2.31 ± 0.13a	3.26 ± 0.02a	3.2 ± 0.1a	13.37 ± 0.65	95.7 ± 0.6b	−2.26 ± 0.06a	18.24 ± 0.55a	18.29 ± 0.47a	97.1 ± 0.4c
	Bentonite 0.5 g/L	0.52 ± 0.01b	3.16 ± 0.02b	0.6 ± 0.1b	2.78 ± 0.12	99.5 ± 0.2a	−0.78 ± 0.02c	2.92 ± 0.16c	3.04 ± 0.18c	105.5 ± 0.3b
SEM/SAB	Control	0.55 ± 0.01b	3.17 ± 0.01b	0.5 ± 0.0b	–	98.6 ± 0.0b	−0.32 ± 0.03b	3.49 ± 0.04b	3.50 ± 0.04b	95.19 ± 0.47a
	GSP 25 g/L	2.74 ± 0.06a	3.29 ± 0.02a	2.5 ± 0.0a	15.83 ± 0.40	94.2 ± 0.3c	−0.46 ± 0.02a	18.68 ± 0.30a	18.69 ± 0.30a	91.41 ± 0.06b
	Bentonite 0.4 g/L	0.51 ± 0.00c	3.18 ± 0.01b	0.5 ± 0.0b	1.23 ± 0.05	99.4 ± 0.0a	−0.23 ± 0.03c	2.54 ± 0.01c	2.55 ± 0.01c	95.26 ± 0.69a
MR	Control	0.67 ± 0.02b	3.28 ± 0.02b	0.4 ± 0.0b	–	98.1 ± 0.0a	0.46 ± 0.12b	3.80 ± 0.02b	3.83 ± 0.04b	81.98 ± 0.07b
	GSP 32 g/L	2.91 ± 0.17a	3.43 ± 0.01a	3.0 ± 0.0a	16.01 ± 0.56	92.4 ± 0.1b	0.50 ± 0.18ab	18.75 ± 0.26a	18.80 ± 0.27a	88.39 ± 0.61a
	Bentonite 1 g/L	0.62 ± 0.00c	3.31 ± 0.01b	0.5 ± 0.1b	0.65 ± 0.08	98.3 ± 0.1a	0.68 ± 0.01a	3.26 ± 0.04c	3.33 ± 0.04c	78.21 ± 0.23c

(Elia *et al.*, 2019)

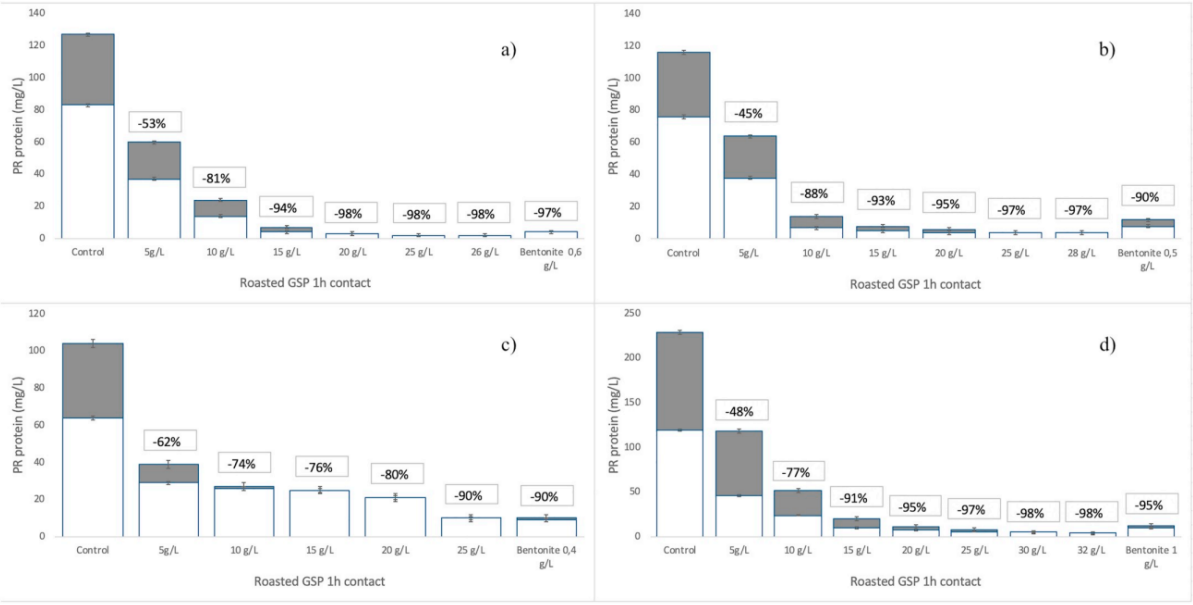


Figure 6. PR protein [TLP (white) + chitinases (grey)] content wines treated with grape seed powder (roasted, 1 h contact) compared to untreated control. a) Fiano (FN), b) Sauvignon blanc (SAB1), c) Semillon/Sauvignon blanc blend (SEM/SAB), d) Marsanne (MR). Percent values represent the decrease of PR protein concentration in treated wines compared to control wines. Values of thaumatin and chitinases are expressed in mg/L thaumatin equivalents and are presented as means and standard deviation of triplicate experiments.

(Elia *et al.*, 2019)

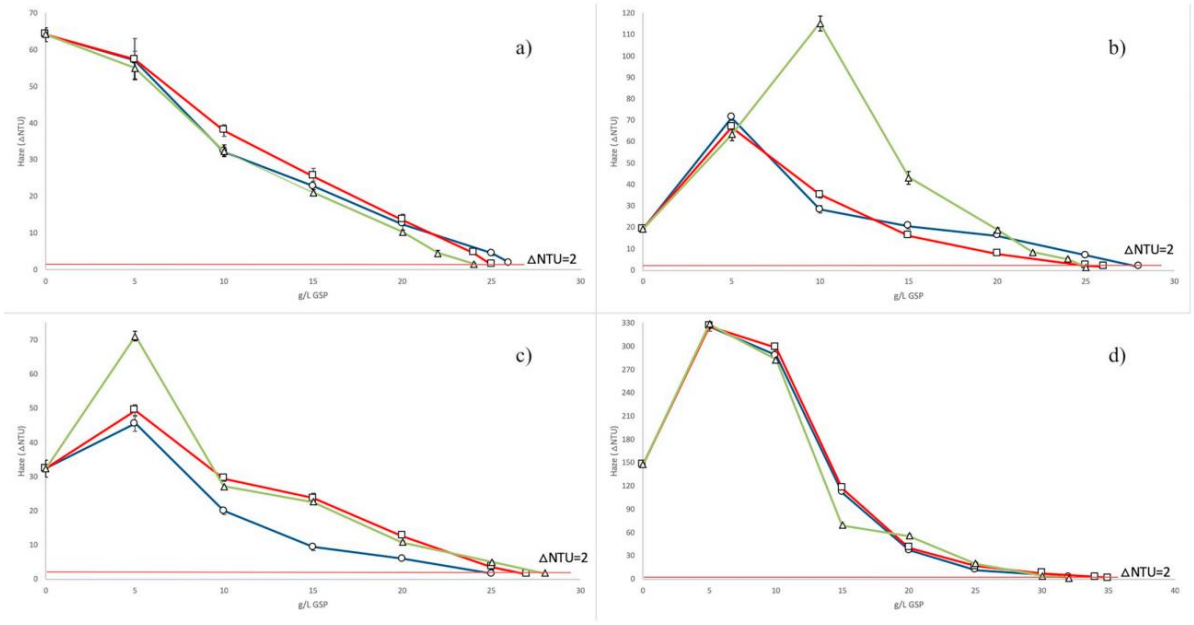


Figure 7. Heat test stability for a) Fiano (FN), b) Sauvignon blanc (SAB1), c) Semillon + Sauvignon blanc (SEM/SAB), d) Marsanne (MR) treated with grape seed powder in different ways: raw, 1 h contact (red, squares); roasted, 1 h contact (blue, circles); roasted,2 h contact (green, triangles). The wines were considered stable when ΔNTU < 2. The values of haze (ΔNTU) are presented as means and standard deviation of triplicate experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Elia *et al.*, 2019)

Table 7. Comparison between wine produced after treatment of juice with 5, 10 and 20 g/L GSP (roasted, 1 h contact) compared with untreated control wines.

Wines	Treatment	A_{280}	pH	Sugars[a]	ΔE	L	a	b	C	H
Semillon	Control	0.58 ± 0.01c	3.26 ± 0.01a	0.1 ± 0.1a	–	97.4 ± 0.1b	0.55 ± 0.10a	7.42 ± 0.20c	7.44 ± 0.19c	85.76 ± 0.89b
	5 g/L GSP	0.63 ± 0.03c	3.28 ± 0.01a	0.0 ± 0.0a	0.66 ± 0.36	97.8 ± 0.4ab	0.53 ± 0.21ab	7.11 ± 0.27c	7.14 ± 0.28c	85.91 ± 1.64b
	10 g/L GSP	0.85 ± 0.01b	3.28 ± 0.01a	0.1 ± 0.0a	2.17 ± 0.43	98.0 ± 0.3a	0.85 ± 0.21a	9.51 ± 0.29b	9.64 ± 0.35b	84.90 ± 0.83b
	20 g/L GSP	1.36 ± 0.07a	3.31 ± 0.02a	0.0 ± 0.1a	5.29 ± 0.61	96.0 ± 0.0c	0.25 ± 0.13b	12.61 ± 0.73a	12.62 ± 0.73a	89.90 ± 2.16a
Sauvignon blanc	Control	0.68 ± 0.00d	3.16 ± 0.01c	0.1 ± 0.1a	–	99.6 ± 0.1a	0.55 ± 0.11a	5.92 ± 0.21c	5.94 ± 0.22c	84.72 ± 1.00b
	5 g/L GSP	0.82 ± 0.02c	3.20 ± 0.01b	0.1 ± 0.1a	1.72 ± 0.71	100.6 ± 0.7a	−0.31 ± 0.71ab	5.79 ± 0.94c	5.83 ± 0.92c	93.78 ± 7.07ab
	10 g/L GSP	1.05 ± 0.04b	3.26 ± 0.01a	0.2 ± 0.1a	2.58 ± 0.63	99.7 ± 0.3a	0.40 ± 0.28a	8.45 ± 0.49b	8.46 ± 0.50b	87.36 ± 1.83b
	20 g/L GSP	1.50 ± 0.03a	3.27 ± 0.01a	0.1 ± 0.0a	4.20 ± 0.08	100.4 ± 0.2a	−1.45 ± 0.04b	9.52 ± 0.32a	9.60 ± 0.29a	98.79 ± 0.49a

Sugars are expressed in g/L of glucose + fructose A280 are expressed in g/L of catechin equivalent. All results are presented as means and standard deviations of triplicate experiments. Different letters in the same column indicate statistically significant ($p < 0.05$) differences for each wine type.

(Elia *et al.*, 2019)

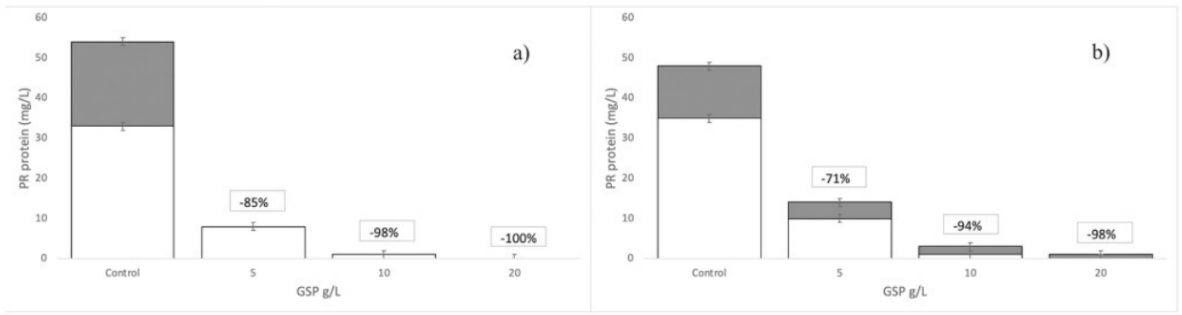


Figure 8. a) PR protein [TLP (white) + chitinases (grey)] content in control and treated Semillon. The % represent the decrease of PR protein of the treated samples compared to the control. The values of thaumatin like protein and chitinases are expressed in mg/L of thaumatin equivalent and are presented as means and standard deviation of triplicate experiments. b) PR protein [TLP (white) + chitinases (grey)] content in control and treated Sauvignon blanc. The % represent the decrease of PR protein of the treated samples compared to the control. The values of thaumatin like protein and chitinases are expressed in mg/L of thaumatin equivalent and are presented as means and standard deviation of triplicate experiments.

(Elia *et al.*, 2019)

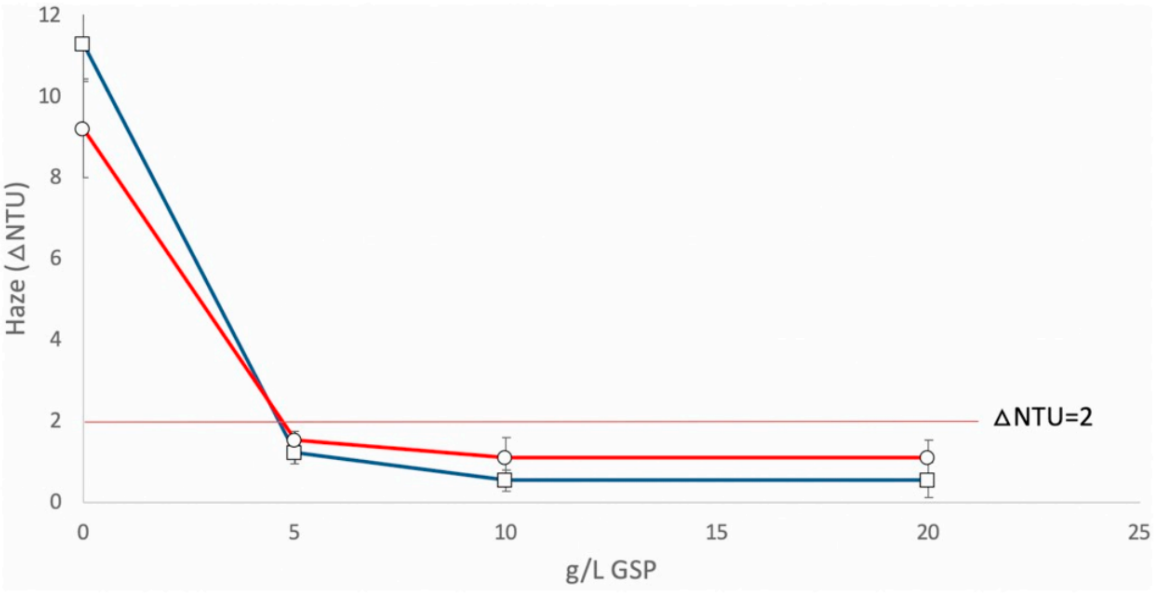


Figure 9. Heat test stability for Semillon (blue, squares) and Sauvignon blanc (red, circles) treated with grape seed powder (roasted, 1 h contact). Wines are considered stable when ΔNTU < 2. Haze (ΔNTU) values are presented as means and standard deviation of triplicate experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Elia *et al.*, 2019)