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RESEARCH

Comparative analysis of pre-fermentation treatments on phenolic compounds and bioactivity in *Vitis Vinifera* var. Syrah and var. Cabernet Sauvignon grapes

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Abstract

This study investigated the effect of pre-fermentation treatments on the phenolic compounds and biological activities of red grapes of *Vitis vinifera* var. Syrah and var. Cabernet Sauvignon Syrah and Cabernet sauvignon musts from two different regions and consecutive vintages. Specifically, cold soak at 10 °C and heat maceration at 60, 70, and 80 °C were compared to classical winemaking (maceration and fermentation at 25 °C). High-performance liquid chromatography/diode array detector (HPLC/DAD) was used to characterize the main classes of polyphenolic substances, and biological activities were determined through assays for antioxidant, anti-inflammatory, anti-hyperuricemic, anti-Alzheimer, anti-diabetic, and anti-proliferative activities. The findings showed that temperature played a significant role in anthocyanin and tannin extraction. The data also revealed moderate positive correlations between dimers procyanidin B1, B2, and caffeic acid with antioxidant activities (ABTS and DPPH), a strong positive correlation between gallic acid and anti-inflammatory activity, another strong positive correlation between gallic acid and anti-diabetic activity, and a moderate correlation between anticancer activity in human breast cancer cells (MCF7) with resveratrol. In conclusion, this study provides valuable insights into the effects of different pre-fermentation treatments on the phenolic composition and biological activities of Syrah and Cabernet sauvignon musts, which may have implications for the development of new winemaking techniques.

Keywords Phenolic compounds · Heat maceration · Syrah · Cabernet Sauvignon · Anti-inflammatory · Anticancer; Anti-diabetic

Introduction

Grapes and wines contain large amounts of phenolic compounds. These compounds are divided into flavonoid and non-flavonoid compounds which contribute greatly to the sensory characteristics of red wine. These compounds include the phenolic acids (p-coumaric, cinnamic, caffeic, gentistic, ferulic, and vanillic acids), trihydroxy stilbenes (resveratrol and polydatin), and flavonoids (catechin, epicatechin, quercetin, anthocyanins, and tannins) [1, 2]. Their specifical chemical structures and their concentrations contribute to their physiological and biological activities [3]. These compounds act as potent antioxidants as they reduce low-density lipoprotein (LDL) cholesterol oxidation, modulate cell signaling pathways, reduce platelet aggregation, inhibit the growth of some tumor types, and exhibit anti-inflammatory, antibacterial, antifungal, antiviral, neuroprotective, antiproliferative, and anti-angiogenic activities [4, 5]. Several studies showed and reviewed the health protective properties of grapes and wine phenolics for several diseases such as some cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, and obesity [4-6]. However, high number of variables such as the presence of alcohol, the complexity of phenolic compounds chemistry, their bioavailability, and human metabolism suggest that conclusion should be drawn carefully. Therefore, the beneficial effects of moderate wine consumption may be attributed to the overall mix of all of its components and not to a specific action of one. But, the consumption of ethanol is debatable in the

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literature because the potential adverse effects of alcohol may outweigh any benefits of phenolic compounds.

The phenolic composition and content of red wine are strongly affected both quantitatively and qualitatively by the particular grape variety, ripeness, environmental factors, and winemaking technological procedures (maceration time and temperature, yeast and enzymes used, SO2 dose, malolactic fermentation, clarification and filtration, ageing) [7, 8]. During the winemaking process, only a fraction of phenolic compounds is transferred from the solid part of the grapes to the liquid affecting strongly the final yield. In order to facilitate the release of phenolic compounds, pre-fermentative maceration conditions have shown to have significant impacts on phenolic and volatile compounds. Therefore, the yield of extraction is temperature dependent within the practical range of 60–80 °C for pre-fermentation heating maceration and 10–15 °C for low temperature [9]. In the literature, it was shown that temperature plays a crucial role in the extraction of phenolic compounds because it influences the permeability of grape cell membranes. It also impacts the viscosity and the density of the solvent. Many strategies are employed in the wine industry in order to facilitate the release of phenolic compounds as thermovinification, pre-fermentation heating maceration, cold maceration, flash release, microwave maceration, ultrasound-assisted extraction, and pulsed electric fields. However, a little is known about the release of phenolic compounds with these strategies and their influence on their chemistry and biological activities.

The purpose of this study was twofold: firstly, to investigate the impact of maceration temperatures on the phenolic composition and biological activities of various red grapes of *Vitis vinifera* var. Syrah and var. Cabernet Sauvignon from two distinct regions and vintages after 24 h of maceration (excluding the effect of ethanol); and secondly, to elucidate possible correlations between phenolic compounds and biological activities using the Pearson correlation coefficient test.

Materials and methods

Chemicals and standards

All chemicals used were of analytical reagent grade. All chromatographic solvents (acetonitrile, acetic acid) were high-performance liquid chromatography (HPLC) grade and were purchased from Sigma-Aldrich (Steinheim, Germany). Delphinidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin 3-*O*-glucoside, (+)—catechin, (-)—epicatechin, (-) -epicatechingallate (-)—epigallocatechin, (-)—epigallocatechingallate, procyanidin B1, procyanidin B2, ferulic acid, caffeic acid, and trans-resveratrol were purchased from Extrasynthèse (Lyon, Genay-France). The Folin-Ciocalteu reagent was obtained from Sigma-Aldrich (Steinheim, Germany).

Grapes

Red grapes of *Vitis vinifera* var. Syrah (Sy) and var. Cabernet Sauvignon (CS) were supplied by two Lebanese cellars from distinct regions: Clos St. Thomas (West Bekaa/Lebanon, annual rainfall of 650 mm, annual average temperature of 21.1 °C) for two consecutive years 2014 and 2015 and Chateau Florentine (Chouf District/Lebanon, annual rainfall of 1078 mm, annual average temperature of 15.1 °C) for 2014. The main characteristics of the samples are presented in Table 1.

Sampling

After reception, the grapes were crushed and destemmed manually and sodium metabisulphite was added (5 g of $NaHSO_3$/100 kg), and a triplicate random of 2 kg of grapes were drawn into glass Erlenmeyer flasks of 2 L sealed with parafilm. To prevent foam overflow, Erlenmeyer flasks were not completely filled. The pomace was manually punched down once daily for macerations and alcoholic fermentations. At the latest 50 mL of each sample was collected and directly centrifuged for 5 min at 5000 rpm. The samples were stored at 0 °C for chemical and biological analyses done one week later.

Microscale maceration process

Pre-fermentative maceration musts were conducted at different temperatures (10, 60, 70, and 80 °C) for the 2014 harvest year and temperatures of 60 and 70 °C for the 2015 harvest year for 24 h without fermentation process. Grapes underwent cold soak maceration with digital temperature controller refrigerator at 10 °C and preheating maceration with a multi-stack shaking incubator (Labtech, LSI-5002 M) at 60, 70, and 80 °C.

Table 1 The grapes samples main characteristics

	2014				2015	
	Sy St Thomas	Sy Florentine	CS St Thomas	CS Florentine	Sy St Thomas	CS St Thomas
°Brix	21.2	23.2	24.2	26.4	22.4	24.2
Titrable acidity g/L (tartaric acid)	6.73	5.66	5.66	4.74	5.51	4.89

Microscale fermentation process

In order to compare the composition and biological activities of the obtained musts, control wines of 2014 Syrah and CS Saint Thomas were made with classical winemaking process (maceration and fermentation steps occurring together at 25 °C). Musts issued from control were separately inoculated by *S. cerevisiae* Y yeast strain kindly provided by Lallemand Inc. (Blagnac, France) at an initial concentration of 3×10^6 cells/mL (Thoma cell counting chamber). The alcoholic fermentation was followed until total or cessation of sugar consumption (< 2 g/L, DNS colorimetric method) and finished after 10 days. Control samples were collected at the end of the alcoholic fermentation. Musts elaborated in each year by cold and preheating macerations were compared with wines made by classical fermentation on skins (control).

Spectrophotometric determinations of polyphenols

Chromatic parameters. The color density (CD) defined as the sum of absorbencies at 420 and 520 [9]. Total anthocyanins (mg/L) were calculated by measurement of the absorbance at 520 nm after bisulfite bleaching solution [9]. Total polyphenol index (TPI) was measured at 280 nm after wine dilution with water (1:100) [9]. Total phenolics (mg gallic acid equivalent/L) were determined using the Folin-Ciocalteu colorimetric method [9]. Total tannins (mg/L) were measured at 550 nm after acid hydrolysis of the samples and a blank [9].

HPLC analysis of phenolic compounds

The HPLC apparatus is a Shimadzu chromatographic system equipped with a quaternary pump (LC-20AD), an UV–Vis diode array detector (SPD-M20A), an automatic injector (SIL-20A), and Shimadzu LC solution software. The method was previously described by [10]. Chromatograms were recorded at 520, 280, and 320 nm for anthocyanins, flavan-3-ols, and phenolics acids respectively. Calibration curves were obtained for all phenols standards and the concentrations were expressed as mg/L.

Determination of biological activities

Samples preparation

Twenty milliliters of musts (after 24 h of maceration) was evaporated to dryness under vacuum using a rotary evaporator (35 °C, 200 rpm). The must extracts were dissolved in dimethyl sulfoxide (DMSO) in order to obtain a final concentration of 50 mg/L in all microplate wells for antioxidant (ABTS and DPPH) assays and a final concentration of 500 mg/L for anti-lipoxygenase (LOX, anti-inflammatory), anti-cholinesterase (ChE, anti-Alzheimer), anti-xanthine oxidase (XOD), anti-α-glucosidase (anti-diabetic), and cytotoxicity activities (anticancer). The total percentage of DMSO in the wells does not exceed 5%.

DPPH-radical scavenging assay (antioxidant activity)

Antioxidant scavenging activity was studied using 1, 1-diphenyl- 2-picrylhydrazyl free radical (DPPH) as described by [11] with some modifications. The absorbance at 524 nm was recorded as A (sample), using UV/Vis microplate spectrophotometer (MultiskanTM GO Thermo Scientific). The free-radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\%\text{inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance of blank}} \times 100 \quad (1)$$

Ascorbic acid was used as the standard. All measurements were performed in triplicate.

ABTS-radical scavenging assay (antioxidant activity)

The radical scavenging capacity of the samples for the ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation was determined. ABTS was produced by mixing 7 mM of ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with water to give an absorbance measurement within the range of 0.7–0.9 at 734 nm using a UV/Vis microplate spectrophotometer (MultiskanTM GO Thermo Scientific). Twenty microliters for each sample was allowed to react with fresh ABTS solution (180 µL), and then the absorbance was measured 6 min after initial mixing. The radical scavenging activity was expressed as percentage of inhibition and calculated in the same way as that previously used for the method of DPPH. Ascorbic acid was used as standard. All measurements were performed in triplicate.

LOX inhibition assay (anti-inflammatory activity)

Lipoxygenase (LOX) is an enzyme that catalyzes the oxidation of unsaturated fatty acids containing 1–4 diene structures. The conversion of linoleic acid to 13-hydroperoxy linoleic acid was followed spectrophotometrically by the appearance of a conjugate diene at 234 nm. LOX was assayed according to the method described by [11], with some modifications.

The absorbance of the resulting mixture was measured at 234 nm using an UV/Vis microplate reader (MultiskanTM GO Thermo Scientific). Inhibition of LOX was calculated using the following equation:

$$\%\ \text{of LOX inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance } of \text{ blank}} \times 100 \quad (2)$$

Nordihydroguaiaretic acid (NDGA) a known inhibitor of soybean lipoxygenase was used as positive control. All determinations were performed in triplicate.

Anti-XOD inhibition assay (anti-hyperuricemic effect)

Determination of xanthine oxidase (XOD) inhibitory activity was evaluated by measuring uric acid production from xanthine or hypoxanthine substrate at 295 nm as described by [11], using a 96-well microplate reader (MultiskanTM GO Thermo Scientific), with some modifications. Inhibition of XOD was calculated as following:

$$\%\ \text{of XOD inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance of blank}} \times 100 \quad (3)$$

Allopurinol was used as a positive control. All determinations were performed in triplicate.

Anti-ChE inhibition assay (anti-Alzheimer activity)

Cholinesterase (ChE) inhibitory activities were measured using Ellman's method [6, 7], with modifications. The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, catalyzed by enzymes at a wavelength of 412 nm. The percentage of inhibition was calculated as following:

$$\%\ \text{of ChE inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance of blank}} \times 100 \quad (4)$$

Galanthaminehydrobromide (GaHBr) was used as positive control. All determinations were performed in triplicate.

α-Glucosidase inhibitory assay (anti-diabetic activity)

The α-glucosidase inhibitory assay was referred to the method of Kim et al. [12] with some modifications. The increase in absorbance due to hydrolysis of PNPG by this enzyme was monitored at 405 nm on a UV/Vis microplate spectrophotometer (MultiskanTM GO Thermo Scientific). The inhibition effect was calculated as follows:

$$\%\alpha - \text{glucosidase inhibition} = \frac{(\text{absorbance of negative control} - \text{absorbance of sample})}{\text{absorbance of negative control}} \times 100 \quad (5)$$

Acarbose was used as a standard inhibitor. All measurements were done in triplicate.

Cytotoxicity assay (antiproliferative activity)

Cytotoxicity of extracts was estimated on human breast cancer (MCF7) and human colon cancer (HCT116) cells as described by Natarajan et al. [13] with modification. Cells were distributed in 96-well plates at 15*103 cells/well in 100 µl of appropriate cell culture medium, and then 100 µl of extract was added, then the mixture was incubated at 37 °C in a CO_2 incubator for 48 h. Cell growth was estimated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells. The resulting blue formazan can be measured spectrophotometrically at 605 nm. The percentage of growth inhibition was calculated according to the following equation:

$$\%\text{of inhibition} = \frac{(\text{absorbance of negative control} - \text{absorbance of sample})}{\text{absorbance of negative control}} \times 100 \quad (6)$$

Tamoxifen was used as positive control. Each extract concentration was tested in triplicate.

Statistical data treatment

All experiments were carried out in triplicate. Pearson's correlation coefficient and Tukey's honestly significant difference (HSD) test were used with a significant level of 95% ($p < 0.05$). These statistical analyses were conducted using Xlstat software (2014).

Results and discussion

Phenolic composition of macerated musts

Table 2 shows the evolution of total anthocyanins, total tannins, total polyphenol, total polyphenol index, color intensity, and the profiles of anthocyanins, flavan-3-ols, and non-flavonoids after 24 h of maceration of Sy and CS musts from the two different regions at different temperatures (10 °C, 60 °C, 70 °C, 80 °C) compared to the control. Table 3 shows the evolution of same parameters during two consecutive vintages (2014 and 2015) at temperatures of 60 °C and 70 °C.

Table 2 Total Anthocyanins, Total polyphenol, Total Tannins, Total Polyphenol Index, Color Intensity Anthocyanins, flavan-3-ols and non-flavonoids profile (mg/L) of 2014 CS and Sy musts from the two distinct regions together with Sy and CS Saint Thomas control in term of time and temperature

	CS and Sy maceration time (hours)-2014						
	24						
		CS-ST Control 25 °C	Sy-ST Control 25 °C	CS-ST	CS-F	Sy-ST	Sy-F
10 °C	TA	187.54 ± 0.50^{b}	220.25 ± 13.47^{a}	161.58 ± 1.23^{b}	179.96 ± 0.54^{a}	43.46 ± 0.5^{b}	51.92 ± 0.31^{a}
	CI	1.20 ± 0.01^{a}	1.22 ± 0.01^{a}	0.46 ± 0.00^{b}	0.55 ± 0.00^{a}	0.52 ± 0.00^{b}	0.59 ± 0.00^{a}
	TPI	50.19 ± 0.04^{b}	60.12 ± 2.57^{a}	19.07 ± 1.66^{a}	17.5 ± 0.95^{a}	20.93 ± 0.94^{a}	15.27 ± 1.62^{b}
	T	1250.35 ± 5.77^{a}	1152.25 ± 46.19^{b}	810 ± 6.41^{a}	726.67 ± 2.92^{b}	663.33 ± 3.09^{a}	540.00 ± 3.22^{b}
	TP	2454.96 ± 22.32^{a}	2332.68 ± 62.14^{b}	5444.62 ± 3.41^{a}	4387.91 ± 3.43^{b}	3344.09 ± 0.69^{a}	3002.59 ± 0.53^{b}
	Dp	4.63 ± 0.30^{b}	6.00 ± 0.18^{a}	0.87 ± 0.013^{b}	0.95 ± 0.01^{a}	n.d	n.d
	Cy	1.91 ± 0.00^{b}	3.12 ± 0.04^{a}	n.d	n.d	n.d	n.d
	Pn	2.92 ± 0.01^{b}	6.10 ± 0.13^{a}	0.11 ± 0.00^{a}	0.29 ± 0.01^{a}	0.17 ± 0.02^{a}	0.28 ± 0.01^{a}
	Mv	66.35 ± 1.98^{a}	65.35 ± 0.51^{a}	20.65 ± 0.11^{a}	19.2 ± 0.05^{a}	1.83 ± 0.01^{a}	1.95 ± 0.02^{a}
	Cat	46.02 ± 0.10^{a}	43.00 ± 0.34^{a}	33.59 ± 0.01^{a}	27.45 ± 0.30^{b}	12.30 ± 0.12^{a}	12.67 ± 0.25^{a}
	Epi	23.25 ± 1.01^{a}	20.22 ± 0.76^{a}	36.23 ± 0.02^{a}	25.66 ± 0.22^{b}	8.58 ± 0.23^{a}	9.67 ± 0.28^{a}
	Epig	23.50 ± 0.36^{a}	22.13 ± 0.89^{a}	4.54 ± 0.01^{a}	3.37 ± 0.02^{a}	6.64 ± 0.11^{a}	10.45 ± 0.39^{a}
	EpiG	42.20 ± 0.04^{a}	39.32 ± 0.29^{a}	28.51 ± 0.05^{a}	17.29 ± 0.05^{b}	8.01 ± 4.88^{a}	4.89 ± 0.58^{b}
	Pro B1	134.10 ± 1.15^{a}	110.05 ± 0.28^{b}	10.40 ± 0.03^{a}	6.2 ± 0.24^{b}	8.34 ± 0.47^{a}	8.21 ± 0.18^{a}
	Pro B2	96.45 ± 1.05^{b}	115.32 ± 0.32^{a}	23.32 ± 0.02^{a}	9.43 ± 0.34^{b}	24.39 ± 0.32^{b}	32.59 ± 1.43^{a}
	GA	22.42 ± 0.17^{a}	23.10 ± 0.10^{a}	1.97 ± 0.01^{a}	0.66 ± 0.00^{b}	2.02 ± 0.04^{a}	1.88 ± 0.03^{a}
	FA	20.15 ± 0.14^{b}	60.22 ± 0.40^{a}	2.85 ± 0.03^{a}	2.44 ± 0.02^{a}	5.68 ± 0.25^{a}	3.72 ± 0.05^{a}
	CA	2.79 ± 0.09^{b}	25.08 ± 0.15^{a}	2.25 ± 0.02^{a}	2.19 ± 0.05^{a}	2.47 ± 0.10^{a}	2.43 ± 0.01^{a}
	Res	7.13 ± 0.09^{a}	7.14 ± 0.00^{a}	1.73 ± 0.01^{a}	0.4 ± 0.00^{b}	1.73 ± 0.04^{b}	2.29 ± 0.09^{a}
60 °C	TA	187.54 ± 0.50^{a}	220.25 ± 13.47^{a}	836.79 ± 0.96^{a}	876.17 ± 3.02^{a}	633.79 ± 0.43^{b}	822.79 ± 0.5^{a}
	CI	1.20 ± 0.01^{a}	1.22 ± 0.01^{a}	1.46 ± 0.02^{b}	2.19 ± 0.03^{a}	1.53 ± 0.01^{b}	1.81 ± 0.16^{a}
	TPI	50.19 ± 0.04^{b}	60.12 ± 2.57^{a}	44.00 ± 1.00^{b}	49.53 ± 2.90^{a}	52.93 ± 1.62^{b}	64.53 ± 1.81^{a}
	T	1250.35 ± 5.77^{a}	1152.25 ± 46.19^{b}	2160.00 ± 2.32^{a}	2120.67 ± 5.23^{a}	2266.67 ± 5.12^{b}	2490.00 ± 0.05^{a}
	TP	2454.96 ± 22.32^{a}	2332.68 ± 62.14^{b}	5859.58 ± 2.14^{b}	6853.97 ± 3.10^{a}	6737.40 ± 1.13^{b}	6443.33 ± 0.48^{a}
	Dp	4.63 ± 0.30^{b}	6.00 ± 0.18^{a}	11.74 ± 0.03^{b}	28.61 ± 0.03^{a}	6.15 ± 0.19^{b}	8.53 ± 0.04^{a}
	Cy	1.91 ± 0.00^{b}	3.12 ± 0.04^{a}	2.42 ± 0.02^{b}	3.86 ± 0.02^{a}	1.62 ± 0.01^{b}	2.47 ± 0.04^{a}
	Pn	2.92 ± 0.01^{b}	6.10 ± 0.13^{a}	4.80 ± 0.04^{b}	8.39 ± 0.03^{a}	10.97 ± 0.01^{a}	10.66 ± 0.02^{a}
	Mv	66.35 ± 1.98^{a}	65.35 ± 0.51^{a}	149.81 ± 0.02^{b}	174.44 ± 0.02^{a}	85.39 ± 0.03^{a}	77.92 ± 0.03^{b}
	Cat	46.02 ± 0.10^{a}	43.00 ± 0.34^{a}	37.89 ± 0.01^{a}	30.38 ± 0.31^{b}	34.21 ± 0.31^{a}	29.62 ± 0.23^{b}
	Epi	23.25 ± 1.01^{a}	20.22 ± 0.76^{a}	34.67 ± 0.01^{a}	32.14 ± 2.18^{a}	41.32 ± 1.20^{b}	57.83 ± 2.11^{a}
	Epig	23.50 ± 0.36^{a}	22.13 ± 0.89^{a}	18.22 ± 0.02^{a}	18.42 ± 0.23^{a}	15.35 ± 0.39^{a}	10.89 ± 2.00^{b}
	EpiG	42.20 ± 0.04^{a}	39.32 ± 0.29^{a}	28.57 ± 0.03^{a}	23.48 ± 1.01^{b}	30.95 ± 0.41^{a}	19.23 ± 3.08^{b}
	Pro B1	134.10 ± 1.15^{a}	110.05 ± 0.28^{b}	112.76 ± 0.03^{a}	104.86 ± 0.16^{b}	102.89 ± 0.53^{b}	115.97 ± 2.86^{a}
	Pro B2	96.45 ± 1.05^{b}	115.32 ± 0.32^{a}	66.84 ± 0.01^{a}	34.10 ± 1.43^{b}	101.24 ± 2.96^{a}	97.85 ± 0.60^{a}
	GA	22.42 ± 0.17^{a}	23.10 ± 0.10^{a}	3.56 ± 0.01^{a}	2.46 ± 0.01^{b}	3.02 ± 0.02^{a}	2.88 ± 0.00^{b}
	FA	20.15 ± 0.14^{b}	60.22 ± 0.40^{a}	14.54 ± 0.01^{a}	10.46 ± 0.31^{b}	18.66 ± 0.43^{a}	15.49 ± 0.42^{b}
	CA	2.79 ± 0.09^{b}	25.08 ± 0.15^{a}	9.19 ± 0.01^{a}	4.23 ± 0.12^{b}	5.73 ± 0.20^{b}	10.64 ± 0.31^{a}
	Res	7.13 ± 0.09^{a}	7.14 ± 0.00^{a}	7.77 ± 0.02^{b}	23.30 ± 0.05^{a}	3.34 ± 0.10^{b}	17.9 ± 0.61^{a}

Table 2 (continued)

	CS and Sy maceration time (hours)-2014						
	24						
		CS-ST Control 25 °C	Sy-ST Control 25 °C	CS-ST	CS-F	Sy-ST	Sy-F
70 °C	TA	187.54 ± 0.50[b]	220.25 ± 13.47[a]	746.67 ± 7.28[a]	752.5 ± 0.61[a]	506.33 ± 1.81[b]	818.42 ± 0.65[a]
	CI	1.20 ± 0.01[a]	1.22 ± 0.01[a]	1.49 ± 0.04[b]	2.08 ± 0.05[a]	1.60 ± 0.02[b]	2.44 ± 0.09[a]
	TPI	50.19 ± 0.04[b]	60.12 ± 2.57[a]	62.73 ± 0.61[a]	64.47 ± 1.47[a]	73.73 ± 2.47[a]	71.80 ± 1.14[a]
	T	1250.35 ± 5.77[a]	1152.25 ± 46.19[b]	3766.67 ± 1.51[a]	3711.67 ± 1.92[a]	3585.00 ± 1.97[a]	3185.00 ± 7.55[b]
	TP	2454.96 ± 22.32[a]	2332.68 ± 62.14[b]	10,853.62 ± 2.45[a]	10,579.95 ± 4.50[b]	9434.32 ± 0.25[a]	8389.22 ± 0.71[b]
	Dp	4.63 ± 0.30[b]	6.00 ± 0.18[a]	18.26 ± 0.03[b]	29.22 ± 0.05[a]	5.53 ± 0.00[b]	9.16 ± 0.04[a]
	Cy	1.91 ± 0.00[b]	3.12 ± 0.04[a]	1.54 ± 0.02[b]	1.98 ± 0.05[a]	1.37 ± 0.01[b]	2.49 ± 0.03[a]
	Pn	2.92 ± 0.01[b]	6.10 ± 0.13[a]	3.59 ± 0.05[b]	5.83 ± 0.01[a]	4.77 ± 0.03[b]	6.87 ± 0.01[a]
	Mv	66.35 ± 1.98[a]	65.35 ± 0.51[a]	82.32 ± 0.05[b]	105.46 ± 0.03[a]	28.73 ± 0.00[b]	37.76 ± 0.05[a]
	Cat	46.02 ± 0.10[a]	43.00 ± 0.34[a]	85.56 ± 0.02[a]	58.90 ± 0.50[b]	45.12 ± 1.47[a]	32.97 ± 1.17[b]
	Epi	23.25 ± 1.01[a]	20.22 ± 0.76[a]	58.59 ± 0.02[a]	51.55 ± 1.02[b]	51.14 ± 1.44[a]	43.40 ± 1.60[b]
	Epig	23.50 ± 0.36[a]	22.13 ± 0.89[a]	32.13 ± 0.02[a]	33.78 ± 1.53[a]	25.52 ± 0.29[a]	22.24 ± 2.46[a]
	EpiG	42.20 ± 0.04[a]	39.32 ± 0.29[a]	35.97 ± 0.05[a]	25.48 ± 2.41[b]	31.66 ± 1.90[a]	22.22 ± 2.23[b]
	Pro B1	134.10 ± 1.15[a]	110.05 ± 0.28[b]	179.59 ± 0.01[a]	175.04 ± 2.89[a]	165.03 ± 0.05[b]	173.4 ± 4.91[a]
	Pro B2	96.45 ± 1.05[b]	115.32 ± 0.32[a]	144.21 ± 0.04[a]	91.34 ± 0.034[b]	134.04 ± 1.34[b]	156.15 ± 0.88[a]
	GA	22.42 ± 0.17[a]	23.10 ± 0.10[a]	4.14 ± 0.01[a]	2.50 ± 0.05[b]	5.50 ± 0.01[a]	3.41 ± 1.34[b]
	FA	20.15 ± 0.14[b]	60.22 ± 0.40[a]	15.19 ± 0.01[a]	11.79 ± 0.13[b]	16.33 ± 0.04[b]	24.02 ± 0.04[a]
	CA	2.79 ± 0.09[b]	25.08 ± 0.15[a]	10.78 ± 0.05[a]	5.52 ± 0.21[b]	12.87 ± 0.08[b]	16.64 ± 0.62[a]
	Res	7.13 ± 0.09[a]	7.14 ± 0.00[a]	15.35 ± 0.01[b]	33.33 ± 0.05[a]	9.57 ± 0.38[b]	37.99 ± 0.72[a]
80 °C	TA	187.54 ± 0.50[b]	220.25 ± 13.47[a]	285.75 ± 1.30[b]	354.37 ± 3.64[a]	234.21 ± 0.11[b]	430.70 ± 0.75[a]
	CI	1.20 ± 0.01[a]	1.22 ± 0.01[a]	1.79 ± 0.05[b]	2.11 ± 0.01[a]	1.93 ± 0.06[a]	2.01 ± 0.17[a]
	TPI	50.19 ± 0.04[b]	60.12 ± 2.57[a]	88.77 ± 3.90[b]	98.07 ± 1.20[a]	85.80 ± 1.15[a]	80.50 ± 0.29[b]
	T	1250.35 ± 5.77[a]	1152.25 ± 46.19[b]	3730.00 ± 2.56[a]	3588.33 ± 4.81[b]	3661.67 ± 0.50[a]	3542.80 ± 1.44[b]
	TP	2454.96 ± 22.32[a]	2332.68 ± 62.14[b]	9806.75 ± 4.62[a]	9072.21 ± 6.48[b]	8498.75 ± 0.09[a]	8105.60 ± 2.71[b]
	Dp	4.63 ± 0.30[b]	6.00 ± 0.18[a]	8.12 ± 0.03[a]	8.82 ± 0.32[a]	n.d	n.d
	Cy	1.91 ± 0.00[b]	3.12 ± 0.04[a]	1.09 ± 0.01[a]	1.07 ± 0.00[a]	n.d	0.60 ± 0.00[a]
	Pn	2.92 ± 0.01[b]	6.10 ± 0.13[a]	0.17 ± 0.01[a]	0.27 ± 0.00[a]	0.15 ± 0.04[b]	0.80 ± 0.00[a]
	Mv	66.35 ± 1.98[a]	65.35 ± 0.51[a]	7.27 ± 0.05[b]	9.25 ± 0.03[a]	3.42 ± 0.01[b]	8.96 ± 0.00[a]
	Cat	46.02 ± 0.10[a]	43.00 ± 0.34[a]	97.12 ± 0.04[a]	83.65 ± 0.83[b]	43.36 ± 0.58[a]	33.28 ± 0.00[b]
	Epi	23.25 ± 1.01[a]	20.22 ± 0.76[a]	105.48 ± 0.02[a]	104.07 ± 0.63[a]	59.73 ± 0.72[a]	48.30 ± 0.00[b]
	Epig	23.50 ± 0.36[a]	22.13 ± 0.89[a]	94.52 ± 0.05[a]	59.32 ± 0.76[b]	27.32 ± 0.03[a]	24.60 ± 4.62[a]
	EpiG	42.20 ± 0.04[a]	39.32 ± 0.29[a]	84.73 ± 0.01[a]	73.3 ± 2.34[b]	37.22 ± 0.16[a]	25.20 ± 2.89[b]
	Pro B1	134.10 ± 1.15[a]	110.05 ± 0.28[b]	154.15 ± 0.05[b]	196.47 ± 2.77[a]	158.79 ± 0.81[b]	178.40 ± 1.85[a]
	Pro B2	96.45 ± 1.05[b]	115.32 ± 0.32[a]	184.58 ± 0.04[b]	197.32 ± 3.37[a]	134.75 ± 2.15[b]	185.30 ± 2.89[a]
	GA	22.42 ± 0.17[a]	23.10 ± 0.10[a]	2.71 ± 0.15[a]	3.87 ± 0.15[a]	6.29 ± 0.11[a]	4.19 ± 0.12[b]
	FA	20.15 ± 0.14[b]	60.22 ± 0.40[a]	14.44 ± 0.04[b]	21.45 ± 0.40[a]	20.02 ± 0.81[b]	24.80 ± 1.73[a]
	CA	2.79 ± 0.09[b]	25.08 ± 0.15[a]	16.64 ± 0.04[a]	7.50 ± 0.22[b]	19.78 ± 0.78[b]	22.80 ± 0.52[a]
	Res	7.13 ± 0.09[a]	7.14 ± 0.00[a]	21.23 ± 0.01[b]	42.24 ± 0.02[a]	16.39 ± 0.41[b]	35.60 ± 0.80[a]

Mean ($n = 3$) ± SD. For each grape variety from the two distinct regions, different letters in the same row indicate significant difference at $p < 0.05$. *TA*, total anthocyanins; *CI*, color intensity; *TPI*, total phenolic index; *TP*, total phenolic; *T*, tannins; *Dp*, delphinidin-3-O-glucoside; *Cy*, cyanidin-3-O-glucoside; *Pn*, peonidin-3-O-glucoside; *Mv*, malvidin-3-O-glucoside; *Cat*, catechin; *Epi*, epicatechin; *Epig*, epicatechin gallte; *EpiG*, epigallocatechin; *Pro B1*, procyanidin B1; *Pro B2*, procyanidin B2; *G.A.*, gallic acid; *F.A.*, ferulic acid; *C.A.*, caffeic acid; *Res*, resveratrol; *CS-ST*, Cabernet Sauvignon Saint Thomas; *CS-F*, Cabernet Sauvignon Florentine; *Sy-ST*, Syrah Saint Thomas; *Sy-F*, Syrah Florentine

Table 3 Total anthocyanins, total polyphenol, total tannins, total polyphenol index, color intensity anthocyanins, flavan-3-ols, and non-flavonoids profile (mg/L) of CS and Sy Saint Thomas musts from the two consecutive vintages at 60 and 70 °C and the 2014 vintage of CS and Sy control (25 °C) in terms of temperature

		CS and Sy-ST maceration time (hours)					
		24					
		CS-ST Control 25 °C	Sy-ST Control 25 °C	CS-ST-014	CS-ST-015	Sy-ST-014	Sy-ST-015
60 °C	TA	187.54 ± 0.50[b]	220.25 ± 13.47[a]	836.79 ± 0.96[a]	290.80 ± 1.30[b]	633.79 ± 0.43[a]	292.20 ± 5.70[b]
	CI	1.20 ± 0.01[a]	1.22 ± 0.01[a]	1.46 ± 0.02[a]	0.91 ± 0.03[b]	1.53 ± 0.01[a]	1.08 ± 0.06[b]
	TPI	50.19 ± 0.04[b]	60.12 ± 2.57[a]	44.00 ± 1.00[a]	38.63 ± 0.51[b]	52.93 ± 1.62[a]	42.00 ± 2.19[b]
	T	1250.35 ± 5.77[a]	1152.25 ± 46.19[b]	2160.00 ± 2.32[a]	2198.33 ± 0.32[a]	2266.67 ± 5.12[a]	2172.67 ± 28.43[b]
	TP	2454.96 ± 22.32[a]	2332.68 ± 62.14[b]	5859.58 ± 2.14[a]	5425.30 ± 135.80[b]	6737.40 ± 1.13[a]	6046.1 ± 133.9[b]
	Dp	4.63 ± 0.30[b]	6.00 ± 0.18[a]	11.74 ± 0.03[a]	1.89 ± 0.01[b]	6.15 ± 0.19[a]	2.01 ± 0.01[b]
	Cy	1.91 ± 0.00[b]	3.12 ± 0.04[a]	2.42 ± 0.02[a]	1.55 ± 0.02[b]	1.62 ± 0.01[b]	2.23 ± 0.06[a]
	Pn	2.92 ± 0.01[b]	6.10 ± 0.13[a]	4.80 ± 0.04[b]	6.31 ± 0.14[a]	10.97 ± 0.01[b]	12.46 ± 0.79[a]
	Mv	66.35 ± 1.98[a]	65.35 ± 0.51[a]	149.81 ± 0.02[a]	95.82 ± 1.11[b]	85.39 ± 0.03[a]	53.42 ± 1.03[b]
	Cat	46.02 ± 0.10[a]	43.00 ± 0.34[a]	37.89 ± 0.01[a]	35.28 ± 0.39[a]	34.21 ± 0.31[b]	56.82 ± 1.08[a]
	Epi	23.25 ± 1.01[a]	20.22 ± 0.76[a]	34.67 ± 0.01[a]	37.28 ± 0.21[a]	41.32 ± 1.20[b]	61.06 ± 1.29[a]
	Epig	23.50 ± 0.36[a]	22.13 ± 0.89[a]	18.22 ± 0.02[a]	10.26 ± 0.02[b]	15.35 ± 0.39[a]	12.53 ± 0.49[b]
	EpiG	42.20 ± 0.04[a]	39.32 ± 0.29[a]	28.57 ± 0.03[a]	23.59 ± 1.12[b]	30.95 ± 0.41[b]	35.82 ± 0.27[a]
	Pro B1	187.54 ± 0.50[b]	110.05 ± 0.28[b]	112.76 ± 0.03[a]	107.50 ± 1.73[a]	102.89 ± 0.53[a]	93.97 ± 0.81[b]
	Pro B2	96.45 ± 1.05[b]	115.32 ± 0.32[a]	66.84 ± 0.01[a]	60.18 ± 2.15[b]	101.24 ± 2.96[a]	82.89 ± 1.56[b]
	GA	22.42 ± 0.17[a]	23.10 ± 0.10[a]	3.56 ± 0.01[b]	6.6 ± 0.07[a]	3.02 ± 0.02[b]	5.16 ± 0.68[a]
	FA	20.15 ± 0.14[b]	60.22 ± 0.40[a]	14.54 ± 0.01[b]	25.39 ± 0.53[a]	18.66 ± 0.43[b]	57.11 ± 1.14[a]
	CA	2.79 ± 0.09[b]	25.08 ± 0.15[a]	9.19 ± 0.01[a]	4.43 ± 0.00[b]	5.73 ± 0.20[a]	4.65 ± 0.10[a]
	Res	7.13 ± 0.09[a]	7.14 ± 0.00[a]	7.77 ± 0.02[a]	3.76 ± 0.04[b]	3.34 ± 0.10[a]	3.40 ± 0.07[a]
70 °C	TA	187.54 ± 0.50[b]	220.25 ± 13.47[a]	746.67 ± 7.28[a]	292.20 ± 0.9[b]	506.33 ± 1.81[a]	331.90 ± 7.10[b]
	CI	1.20 ± 0.01[a]	1.22 ± 0.01[a]	1.49 ± 0.04[a]	1.26 ± 0.01[b]	1.60 ± 0.02[a]	1.46 ± 0.09[b]
	TPI	50.19 ± 0.04[b]	60.12 ± 2.57[a]	62.73 ± 0.61[a]	56.60 ± 2.07[b]	73.73 ± 2.47[a]	60.47 ± 1.97[b]
	T	1250.35 ± 5.77[a]	1152.25 ± 46.19[b]	3766.67 ± 1.51[a]	3180 ± 1.02[b]	3585 ± 1.97[a]	3468.33 ± 2.88[b]
	TP	2454.96 ± 22.32[a]	2332.68 ± 62.14[b]	10853.62 ± 2.45[a]	7377.7 ± 2936.4[b]	9434.32 ± 0.25[a]	8746.2 ± 165.1[b]
	Dp	4.63 ± 0.30[b]	6.00 ± 0.18[a]	18.26 ± 0.03[a]	2.27 ± 0.01[b]	5.53 ± 0.00[a]	2.14 ± 0.01[b]
	Cy	1.91 ± 0.00[b]	3.12 ± 0.04[a]	1.54 ± 0.02[a]	1.41 ± 0.04[b]	1.37 ± 0.01[b]	2.71 ± 0.05[a]
	Pn	2.92 ± 0.01[b]	6.10 ± 0.13[a]	3.59 ± 0.05[b]	4.87 ± 0.04[a]	10.77 ± 0.03[b]	12.51 ± 0.25[a]
	Mv	66.35 ± 1.98[a]	65.35 ± 0.51[a]	82.32 ± 0.05[a]	54.54 ± 1.41[b]	28.73 ± 0.00[a]	10.65 ± 2.05[b]
	Cat	46.02 ± 0.10[a]	43.00 ± 0.34[a]	85.56 ± 0.02[a]	55.77 ± 0.54[b]	45.12 ± 1.47[a]	32.72 ± 0.64[b]
	Epi	23.25 ± 1.01[a]	20.22 ± 0.76[a]	58.59 ± 0.02[b]	87.24 ± 2.76[a]	51.14 ± 1.44[a]	55.33 ± 2.72[a]
	Epig	23.50 ± 0.36[a]	22.13 ± 0.89[a]	32.13 ± 0.02[a]	23.04 ± 0.43[b]	25.52 ± 0.29[a]	13.36 ± 2.22[b]
	EpiG	42.20 ± 0.04[a]	39.32 ± 0.29[a]	35.97 ± 0.05[a]	25.08 ± 2.23[b]	31.66 ± 1.90[a]	30.74 ± 2.47[a]
	Pro B1	187.54 ± 0.50[b]	110.05 ± 0.28[b]	179.59 ± 0.01[a]	154.70 ± 3.97[b]	165.03 ± 0.05[a]	161.05 ± 1.14[a]
	Pro B2	96.45 ± 1.05[b]	115.32 ± 0.32[a]	144.21 ± 0.04[a]	124.57 ± 3.18[b]	134.04 ± 1.34[a]	124.21 ± 0.64[b]
	GA	22.42 ± 0.17[a]	23.10 ± 0.10[a]	4.14 ± 0.01[b]	6.98 ± 0.14[a]	5.50 ± 0.01[a]	6.45 ± 0.22[a]
	FA	20.15 ± 0.14[b]	60.22 ± 0.40[a]	15.19 ± 0.01[b]	27.22 ± 0.65[a]	16.33 ± 0.04[a]	10.20 ± 2.75[b]
	CA	2.79 ± 0.09[b]	25.08 ± 0.15[a]	10.78 ± 0.05[a]	9.66 ± 0.05[a]	12.87 ± 0.08[a]	9.45 ± 0.20[b]
	Res	7.13 ± 0.09[a]	7.14 ± 0.00[a]	15.35 ± 0.01[a]	5.82 ± 0.05[b]	9.57 ± 0.38[a]	6.84 ± 0.03[b]

Mean ($n = 3$) ± SD. For each grape variety from the two consecutive vintages (2014 and 2015), different letters in the same row indicate significant difference at $p < 0.05$. *TA*, total anthocyanins; *CI*, color intensity; *TPI*, total phenolic index; *TP*, total phenolic; *T*, tannins; *Dp*, delphinidin-3-O-glucoside; *Cy*, cyanidin-3-O-glucoside; *Pn*, peonidin-3-O-glucoside; *Mv*, malvidin-3-O-glucoside; *Cat*, catechin; *Epi*, epicatechin; *Epig*, epicatechin gallte; *EpiG*, epigallocatechin; *Pro B1*, procyanidin B1; *Pro B2*, procyanidin B2; *G.A.*, gallic acid; *F.A.*, ferulic acid; *C.A.*, caffeic acid; *Res*, resveratrol; *CS-ST*, Cabernet Sauvignon Saint Thomas; *Sy-ST*, Syrah Saint Thomas; *n.d.*, not detected values

The results showed that temperature affects the amounts of tannins and anthocyanins released from skins and seeds to the must. Maceration at 60 °C allowed obtaining the maximum of total anthocyanins for both grape varieties from two different regions while the maximum of total tannins and total polyphenols were obtained when macerating at 70 °C. The maximums values obtained for total anthocyanins, total tannins, and total polyphenols are higher than those obtained by the control (maceration + fermentation in the same time). For temperatures of 70 °C and 80 °C, a significant decrease in total anthocyanins was observed for the different grape musts compared to 60 °C. This decrease is much greater for 80 °C than for 70 °C where concentrations were divided by an average factor of 2.5 and 1.07 while macerating at 80 °C and 70 °C respectively. This decrease of anthocyanins has been attributed to multiple factors such as heat degradation, oxidative cleavage leading to anthocyanin degradation, copigmentation or reaction with other wine components, and formation of pyranoanthocyanins [9]. When comparing the 2 vintages, Sy and CS musts of 2014 vintage showed respectively 1.5 to 2.8 times higher anthocyanin contents and 1.25 to 1.8 times higher tannin contents than the 2015 vintage after 24 h of maceration (Table 3).

Heating not only increased the total anthocyanins concentration, but also led to an increase of color intensity. For the different grape musts and vintages, a gradual increase in color intensity was observed with increasing maceration temperature up to 80 °C (Tables 2 and 3). This increase in CI associated with a decrease in total anthocyanins can be explained by the formation of new compounds due to copigmentation and condensations reactions [9]

The total polyphenols are characterized qualitatively by the total polyphenols index (TPI) and quantitatively by the analysis of the total polyphenol (TP) by the Folin-Ciocalteu method. The results showed an increase in TPI and TP with temperature. The increase of phenolic compounds with increasing maceration temperature (Tables 2 and 3) can be explained by the fact that the heat destroys the skin's cell membranes, releasing the pigments, tannins, and different phenolic substances into the must [8, 9].

Concerning the anthocyanins profile, results showed that the maceration temperature highly influenced the anthocyanins monomers profile. At 10 °C, malvidin-3-O-glucoside was the most abundant compound found at this temperature with a maximum concentration of 20.7 mg/L for CS from the two regions. Increasing the maceration temperature led to an increase in the concentrations of anthocyanins monomers. The maximum concentrations for all anthocyanins' compounds were obtained at 60 °C. The maceration at 80 °C showed a drastic degradation of the anthocyanins monomers. Eventually, monomeric anthocyanins detected by HPLC showed similar tendency than total anthocyanins analyzed by spectrophotometer. With few exceptions, 2014 vintage from the two grape varieties showed significantly higher anthocyanins profiles than 2015.

Concerning monomeric and dimeric tannins from the two different regions and vintages, their extraction is favored by higher temperatures (Tables 2 and 3). In terms of concentration, catechin and epicatechin are the most represented monomer of flavan-3-ols. With few exceptions, 2015 vintage of Sy and CS musts showed significantly higher values of catechin and epicatechin for the different maceration temperatures than for those of 2014 vintage. Unlike anthocyanins, tannins monomers seemed to resist thermal degradation where the optimum extraction temperature is 80 °C. Among grape varieties and vintages, 2014 vintage of CS and Sy showed significantly higher values of dimeric tannins (Pro B1 and B2) than for 2015 (Table 2). For oligomers (Pro B1 and B2), studies showed that skin's dimeric proanthocyanidins are preferentially extracted during the early stages of maceration [8]. The diffusion of dimers follows extraction kinetics to those reported for skin's flavan-3-ols.

Concerning the phenolic acids, results obtained from Tables 2 and 3 showed that heat promotes gallic, caffeic, and ferulic acid extraction compared to low temperature (10 °C). These phenolic acids had higher maximums at 80 °C. By comparing the results obtained to the control, with few exceptions, Tables 2 and 3 show that Syrah control exhibited higher values of phenolic acids compared to Syrah musts macerated at different temperatures and vintages after 24 h. After all, 2014 vintage of the different musts showed significantly higher values of caffeic acid whereas 2015 vintage showed significantly higher values of gallic and ferulic for the different temperatures and grape varieties (Tables 2 and 3).

Regarding stilbenes, the highest level of resveratrol is obtained by macerating at 80 °C for 24 h (42.24 mg/L, CS-F-2014) without any detection of degradation. 2014 vintage of the different musts showed significantly higher values of resveratrol which is on average value almost twice higher than for the 2015 musts.

Biological activities of macerated musts: correlation between biological activities and phenolic compounds in terms of time and temperature

By comparing the different biological activities found in the different grape must varieties at two consecutives vintages after 24 h of maceration at different temperatures (10 °C, 60 °C, 70 °C, 80 °C, and 25 °C), Fig. 1A illustrates that Sy musts macerated at 10 °C and 60 °C showed low biological activities compared to 70 °C and 80 °C. Sy-ST-2014 macerated at 70 °C exhibited the highest inhibition percentage for the most of the biological activities studied. The ABTS, DPPH, LOX, α-glucosidase, and HCT116 values were respectively 63.3, 52.4, 84.6, 35.7, and 47.6%. These values are 3 times

higher for ABTS and DPPH, 18 times higher for LOX, and 2.21 times higher for HCT116 than for Sy-F at the same temperature. However, results for CS musts did not show any significant biological activity whatever the maceration temperature (Fig. 1B) except for the control. CS-ST-70 °C showed the highest inhibition's percentage for ABTS, DPPH, LOX, and α-glucosidase (24.19, 19.15, 27.21, 9.49; 5.98% respectively), but these values were 2.65 times lower for ABTS and DPPH, 3.10 times lower for LOX, 3.76 times lower for α-glucosidase, 1.11 times lower for ChE, and 14.42 times lower for HCT116 than for Sy-ST. For both CS and Sy musts, control showed significant anti-LOX and anti-α-gluc activities.

Figure 2A and B shows the results of biological activities of Saint Thomas Sy and CS musts macerated at 60 and 70 °C for two consecutive years. Results showed that Sy-ST-70 °C-2014 presented the highest biological activities in terms of antioxidant (ABTS and DPPH) and anti-inflammatory (anti-LOX) activities. Besides, Sy-ST-70 °C-2014 showed percentage of inhibition 2.10, 3, and 2.13 times higher respectively for ABTS, DPPH, and LOX than for Sy-ST-70 °C-2015, whereas anti-α-glucosidase and anti-ChE activity percentage inhibition value was almost the same for the two vintages (Fig. 2A). As for CS musts, Fig. 2B shows that CS-60 °C-2015 presented slightly higher values of ABTS and DPPH than CS-60 °C-2014, while this latter presented percentage inhibition value of approximately 7% respectively for LOX and ChE activity. In other hand, CS-70 °C-2014 presented higher values of ABTS, DPPH, and LOX and same values of anti-α-glucosidase than CS-70 °C-2015.

Over the last three decades, scientific research has point out the positive effects of grape and wine polyphenols on human health through exerting antioxidant capacity scavenging reactive oxygen species, decreasing incidence of cardiovascular disease, preventing the development of diabetes, exhibiting anti-inflammatory activities altering the expression of genes like proinflammatory cytokines, lipoxygenase, and nitric oxide synthase, and inhibiting cancer cell proliferation and angiogenesis process [14]. Polyphenols have been shown to exhibit the antioxidant activity through different mechanisms such as scavenging of free radicals or reactive oxygen species, metal

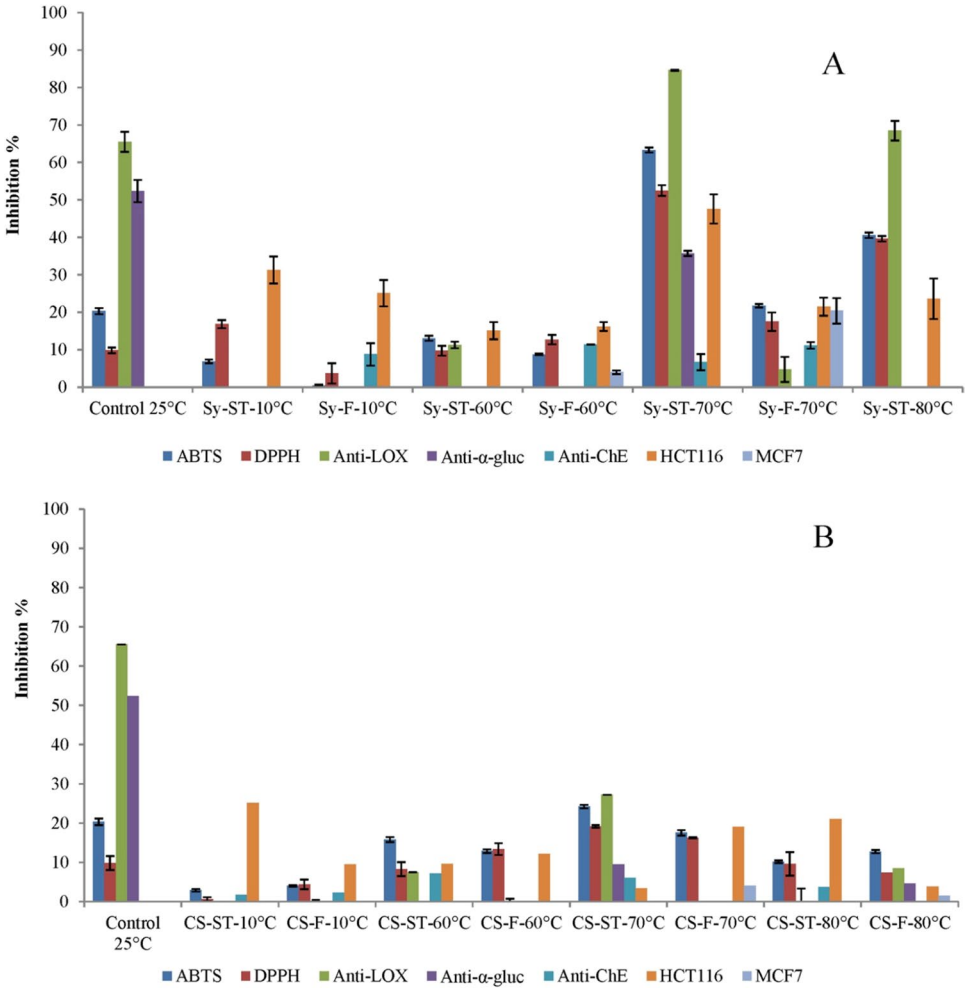

Fig. 1 Biological activities (ABTS and DPPH (antioxidant), anti-LOX (anti-inflammatory), anti-α gluc (anti-diabetic), anti-ChE (anti-Alzheimer), HCT116 and MCF7 (anticancer)) of grape musts from the 2014 vintage macerated at different temperatures (10 °C, 60 °C, 70 °C, 80 °C) after 24 h and for the control (Sy-ST-25 °C) and (CS-ST-25 °C) after alcoholic fermentation. **A** Sy Saint Thomas (Sy-ST) and Sy Florentine (Sy-F). **B** CS Saint Thomas (CS-ST) and CS Florentine (CS-F). Data were expressed as mean ($n=3$) percentage of inhibition (inhibition %) ± standard deviation

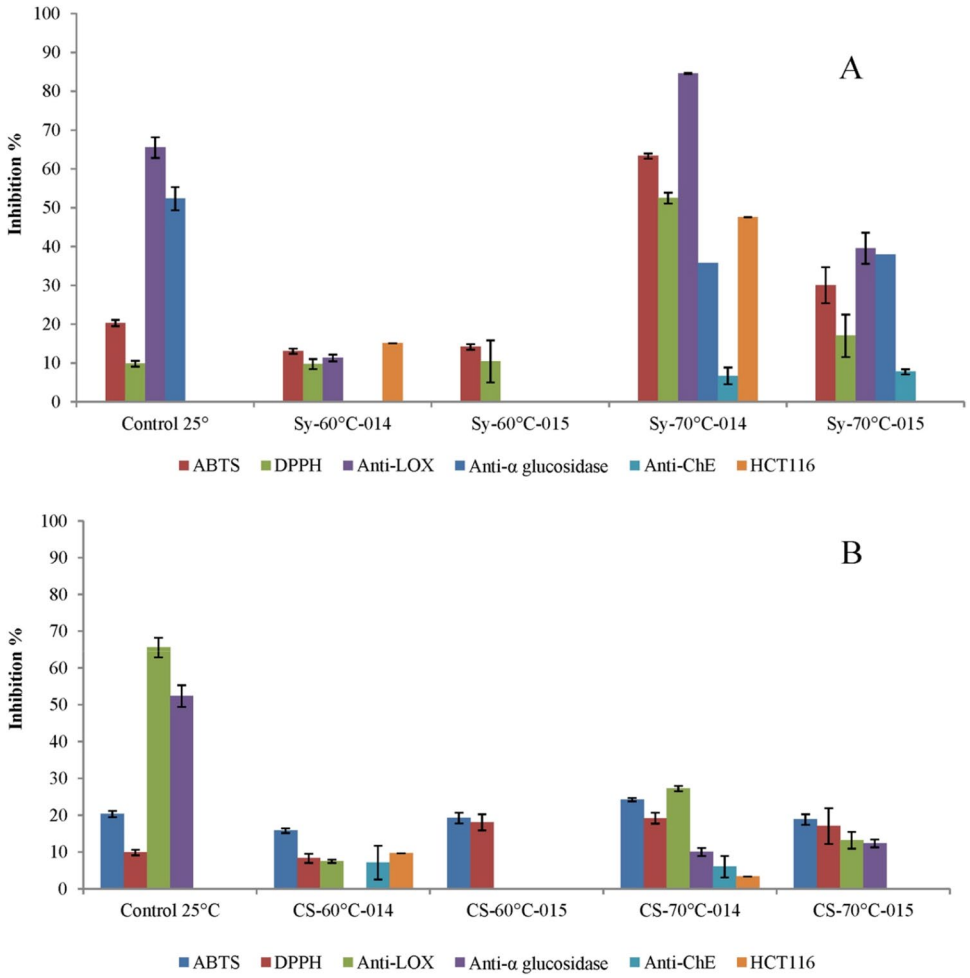

Fig. 2 Biological activities (ABTS and DPPH (antioxidant), anti-LOX (anti-inflammatory), anti-α gluc (anti-diabetic), anti-ChE (anti-Alzheimer)) of grape musts from the 2014 and 2015 vintage macerated at different temperatures (60 °C, 70 °C) after 24 h and for the control (Sy-ST-25 °C) and (CS-ST-25 °C) after alcoholic fermentation. **A** Sy Saint Thomas 2014 and 2015 (Sy-014, Sy-015). **B** CS Saint Thomas 2014 and 2015 (CS-014, CS-015). Data were expressed as mean ($n=3$) percentage of inhibition (inhibition %) ± standard deviation

chelation, inhibition of pro-oxidant enzymes, and activation of antioxidant enzymes [15]. Ky and Teissedre [16] showed that Syrah grape pomace and seed pomace extracts expressed high antioxidant potential through ABTS and DPPH tests. Studies by Lucena et al. [17] showed that Syrah wines from Brazil exhibited high antioxidant activity through ABTS test (CE50 = 1.6 ± 0.03 µg/mL). Some evidences from the literature suggested that polyphenols are beneficial agents to reduce the risk of diabetes and diabetic complications [18]. Polyphenols anti-diabetic effects can be summarized by anti-inflammatory and antioxidant effects, inhibition of digestion enzymes (α-amylase and α-glucosidase), and improvement of insulin resistance, as well as protection of pancreatic cells against glucose toxicity [18]. Dudoit et al. [18] investigated the α-glucosidase inhibitory effect of seed and skin Tannat grape extracts at four ripening stages. They found that skin and seed extracts at the first stage of ripening exhibited strong α-glucosidase inhibition. In our study, moderate α-glucosidase inhibitory effect was recorded for the control samples and for Sy-ST macerated at 70 °C. The α-glucosidase inhibitory effect seems to be impacted by the grape variety, the extraction method used, the ripening stage, and the winemaking scheme. Wine polyphenols inhibitory effect against pro-oxidant enzymes such as lipoxygenase (LOX) has been reported [19] This pro-oxidant enzyme is involved in inflammatory process since it plays an important role in the biosynthesis of inflammatory lipid mediators such as leukotrienes and prostaglandins and its inhibition is considered one of the targets for the prevention of diseases. In the present study, Syrah grapes macerated at 70 and 80 °C showed strong inhibition against lipoxygenase activity. Grape and wines polyphenols have drawn an increased attention for their potential anticancer effects which have been demonstrated in vitro and in vivo models on several cancer types [20]. Antioxidant, anti-inflammatory, and antiproliferative activities of grapes and wine polyphenols have been proposed as mechanisms of potential anticancer effects [21]. Many studies have been focused on the antiproliferative effect of wine polyphenols [22, 23]. In our findings, Syrah grapes macerated at 70 °C showed the highest against human colon cancer cells (HCT117) but no activity was found against human breast cancer cells. Oliveira et al. [24] showed that

Porto wines exhibited an antiproliferative effect against colon cancer cells (Caco-2 and HT-29 cells).

Coefficients of determination were calculated between biological activities and individual phenolic compounds measured for the different grape varieties and vintages macerated at different temperatures (Table 4). So, as seen in Table 4, some individual anthocyanins and stilbenes, like cyanidin and trans-resveratrol, did not provide a contribution to antioxidant activities (0.1634 and 0.0922, respectively for ABTS assay). Significantly higher values found for total polyphenols ($r^2 = 0.4323$), procyanidin B1 ($r^2 = 0.5948$), caffeic acid ($r^2 = 0.4770$), procyanidin B2 ($r^2 = 0.4731$), and epigallocatechin ($r^2 = 0.4305$) evaluated by ABTS assay. Another moderate correlation was found between total polyphenol ($r^2 = 0.4174$), procyanidin B1 ($r^2 = 0.4687$), caffeic acid ($r^2 = 0.3868$), procyanidin B2 ($r^2 = 0.3502$), and antioxidant capacity evaluated by DPPH assay.

Moreover, strong correlation was found between gallic acid ($r^2 = 0.6648$) and anti-inflammatory activity and moderate positive correlation was found between caffeic acid ($r^2 = 0.5130$), procyanidin B1 ($r^2 = 0.3667$), procyanidin B2 ($r^2 = 0.3525$), and anti-inflammatory activity, while weak correlation was found between antiproliferative activity in human colon cancer cells (HCT116) and the main classes of phenolic compounds included this study (Table 4). In addition to that, Sy-F-70 °C indicated 20.4 times higher value of MCF7 than for Sy-ST-70 °C which can be the result of the moderate correlation between anticancer activity in human breast cancer cells (MCF7) with resveratrol ($r^2 = 0.5884$), Furthermore, Sy-ST control had 1.46 times higher anti-diabetic activities than Sy-ST macerated at 70 °C; this can be due as seen in Table 4 to the anti-diabetic strong correlation with gallic acid ($r^2 = 0.8462$) and moderate correlation with caffeic acid ($r^2 = 0.3133$). After all, as seen from Tables 1, 2, and 4) and Figs. (1, 2), compounds with higher contribution to their relative biological activities were found in higher concentrations and must grapes macerated at 70 °C for 24 h presented higher percentage and different types of biological activities for whatever the grape variety and the vintage. So, this could be explained by the fact that not all phenolics compounds had the same contribution to the antioxidant activity. In these regard, Kerry and Abbey [25] demonstrated that the antioxidant characteristics of red wines are due predominantly to monomeric catechins, procyanidins, monomeric proanthocyanidins, and phenolic acids. In addition, Lingua et al. [26] demonstrated that in the case of grapes, astilbin and procyanidin dimers were compounds with highest positive contribution to the FRAP, ABTS, and DPPH values, while peonidin-3-coumaroylglucoside, (-)-epicatechin and myricetin were the ones with highest negative contribution. Important antioxidant components of red wines which include caffeic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, myricetin, protocatechuic acid, quercetin, and resveratrol have been demonstrated with in vitro systems, in cell

Table 4 Pearson's correlation coefficients between biological activities and individual phenolic compounds in Sy and CS musts and controls from two different wine grape growing-regions and vintages macerated at different temperatures

Variables	ABTS	DPPH	LOX	Anti-ChE	α-glucosidase	HCT	MCF7
TA	0.1585	0.1201	−0.1611	**0.3758**	−0.2245	0.0104	**0.4266**
CI	**0.3644**	**0.3209**	0.1152	0.2054	−0.0497	0.0273	**0.4955**
TPI	**0.5329**	**0.4600**	**0.4008**	0.0527	0.1609	0.0070	0.2291
TP	**0.4323**	**0.4174**	0.0670	0.2361	−0.1637	0.0951	0.1476
T	**0.5765**	**0.5465**	0.1888	0.1295	−0.0375	0.0005	0.2195
DP	0.0028	−0.0299	−0.1798	−0.0377	−0.1356	−0.0433	0.1710
CY	0.1634	−0.0209	0.0878	0.1478	**0.3378**	**−0.4260**	0.2317
Pn	0.1619	0.0572	−0.0404	0.1901	0.1264	**−0.3408**	0.1577
Mv	−0.0566	−0.1239	−0.1665	−0.0924	−0.0783	**−0.3109**	−0.0266
Cat	0.1539	0.0400	0.0557	−0.1999	0.0416	−0.2479	−0.0788
Epi	0.1803	0.1466	−0.0620	−0.0387	−0.1472	−0.1515	0.0209
Epig	0.0987	0.0503	0.0425	−0.0606	0.0016	0.0218	0.0180
EpiG	0.1247	−0.0253	0.1725	−0.2226	0.1652	−0.1905	−0.1212
Pro B1	**0.5948**	**0.4687**	**0.3667**	0.0792	**0.2586**	−0.1988	**0.2797**
Pro B2	**0.4731**	**0.3502**	**0.3525**	0.1468	**0.2529**	−0.1050	**0.2738**
GA	0.2400	0.0220	**0.6648**	**−0.2617**	**0.8462**	**−0.4153**	−0.1286
FA	0.0558	−0.0593	0.1051	−0.2166	0.1620	**−0.3244**	0.0260
CA	**0.4770**	**0.3868**	**0.5130**	0.1977	**0.3133**	0.0398	**0.2607**
Res	0.0922	0.0851	−0.1219	0.1496	−0.1919	0.0554	**0.5884**

ABTS, DPPH (antioxidant activity), anti-LOX (anti-inflammatory activity), anti-ChE (anti-Alzheimer activity), anti-α-glucosidase (anti-diabetic activity), HCT and MCF7 (anticancer activity). Values in bold are significant at the 0.05 level

culture, and in human subjects [26]. Some of our findings are consistent with these studies and other appears to be inconsistent. In fact, the antioxidant content and capacity of wine was suggested to depend on the grape variety, the vineyard, the age, and the "terroir." Furthermore, other natural antioxidants presented in the grapes especially viniferin, quercetin, and catechin also inhibit various cyclooxygenase enzymes, which play an important role in inflammatory disorders [27]. Moreover, resveratrol, quercetin, catechins, and anthocyanins have been shown to inhibit hyperglycemia, improve beta-cell function, and protect against beta-cell loss, in type 2 diabetic subjects [28]. Besides, resveratrol suppresses proliferation of a wide variety of tumor cells, including lymphoid, myeloid, breast, prostate, stomach, colon, pancreas, thyroid, skin, head and neck, ovarian, and cervical [1].

Conclusions

In conclusion, this study provides valuable insights into the impact of maceration conditions on the phenolic composition and biological activities of grape musts. The results showed that higher temperatures and longer maceration times result in a greater concentration of total anthocyanins, with the highest concentration observed at 70 °C for 24 h. This is of particular interest to wine makers, who may be able to use this information to develop new winemaking techniques that optimize the extraction of phenolic compounds for enhanced color, flavor, and health-promoting properties. The HPLC analysis revealed that malvidin-3-*O*-glucoside, catechin, and epicatechin were the major anthocyanins and flavan-3-ols present in the musts. These results provide a basis for future studies examining the contribution of these individual compounds to the sensory and health properties of wine. The biological activity analysis revealed that the must macerated at 70 °C for 24 h had the highest percentage and variety of biological activities compared to musts macerated at other temperatures. This finding suggests that the maceration conditions can have a significant impact on the health-promoting properties of wine, and provides a basis for future research exploring the health benefits of wine. The Pearson correlation analysis showed that the antioxidant activity was mainly correlated with procyanidin B1, anti-inflammatory activity was positively correlated with gallic and caffeic acid, and anticancer activity in human breast cancer cells was moderately positively correlated with resveratrol. Additionally, a strong positive correlation was found between anti-diabetic activity and gallic acid content. These findings provide valuable insights into the mechanisms underlying the biological activities of wine, and suggest that individual phenolic compounds may play a key role in mediating the health-promoting effects of wine.

Overall, this study highlights the importance of the maceration step in determining the phenolic composition and biological activities of wine, and provides a foundation for future studies exploring the relationships between maceration conditions, phenolic composition, and health properties. It is hoped that this research will contribute to the development of new winemaking techniques that optimize the sensory and health properties of wine, and provide a basis for future studies examining the role of wine in promoting human health.

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Data availability The datasets generated and/or analyzed during the current study are included in the manuscript. Moreover, the datasets used to generate figures and results are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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