Phytoprostanes fingerprinting is different depending on viticulture and enological process in must and wine.

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Dependency of Phytoprostane Fingerprints of Must and Wine on Viticulture and Enological Processes

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ABSTRACT

Wine is one of the most consumed alcoholic samples around the world. Red wine has demonstrated several benefits for health maintenance. One group of potential anti-inflammatory compounds is the Phytoprostanes, oxidative degradation products of linolenic acid. The aim of the present study was to measure, for the first time, the Phytoprostanes content in wine and must by an UHPLC-QqQ-MS/MS method after solid-phase extraction. The data showed two predominant classes of Phytoprostanes: F_1 and D_1-Phytoprostanes series. In wines, the total Phytoprostanes concentration ranged from 134.15±2.33 ng/mL to 216.23±3.06 ng/mL. Musts showed concentrations between 21.43±0.86 ng/mL and 447.1±15.88 ng/mL. The vinification and aging procedures for the production of wine seem to influence the final Phytoprostanes levels in red wine and to modify the Phytoprostanes profile. The high concentrations observed and previous reports on anti-inflammatory effects of Phytoprostanes make further research on the benefits of Phytoprostanes more important.

KEYWORDS

Phytoprostanes, oxidative stress, inflammation, red wine, lipid peroxidation.

INTRODUCTION

Wine is a distinctive component of the Mediterranean diet, and one of the most consumed alcoholic samples in Spain. Wine and grape berries have been demonstrated to provide several benefits for health maintenance.[1]

The main characteristic of wine proposed to benefit health is its ability to scavenge pro-oxidant species such as reactive oxygen species (ROS). The ROS are responsible for a high variety of dysfunctions, notably a dysfunction of the normal physiological function in plants and humans, leading to the oxidation of different molecules, such as amino acids, DNA, or lipids.[2] Lipid peroxidation products are formed by the non-enzymatic oxidation of lipids, such as prostanoids. Arachidonic acid (C20:4ω6) is the most common fatty acid in mammals and it can be converted into prostaglandins by cyclooxygenase, or it can be oxidized to isoprostanes (IsoPs) by free radical-mediated peroxidation. Linolenic acid (C18:3ω3) is widely distributed in the plant kingdom, and can be converted into jasmonic acid by enzymatic reactions.[3] Imbusch and Mueller [4] revealed a new class of dinor isoprostanes in plants, resulting from the non-enzymatic oxidation of linolenic acid, which were named Phytoprostanes (Figure 1). The effects of Phytoprostanes depend on the stereoisomer.[5] Non-enzymatic oxidation of linolenic acid leads to two regioisomeric classes of Phytoprostanes, and each one of these includes 16 isomers which are thought to be synthesized from membrane lipids of plant cells, such as IsoPs in mammals.[3] Peroxidation of linolenic acid results in G_1-Phytoprostanes isomers, which can give rise to D_1, E_1, and F_1-Phytoprostanes. In turn, D_1 and E_1 rings can be converted into J_1 and deoxy-J_1 or A_1 and B_1 rings, respectively.

Regarding the beneficial effects of Phytoprostanes, biological activities have been described in plants.[3] In fact, B_1 and A_1-Phytoprostanes regulate gene expression in *Arabidopsis thaliana* and tobacco.[6-8] In animal models, Phytoprostanes have also demonstrated bioactive effects. For example, A_1 and dJ_1-Phytoprostane, at similar concentration, showed anti-inflammatory effects like those of PGA_1 and $dPGJ_2$. Moreover, E_1-Phytoprostane inhibits *in vitro* synthesis of dendritic cell interleukin-12 and interleukin-1.[9, 10] Concurrently, E_1 and F_1-Phytoprostane were able to reduce the *in vivo* production of cytokine Th1 and Th2 profiles.[11]

Currently, our knowledge of Phytoprostanes quantitation and identification is limited to tobacco, the leaves of some plant species, and tomato.[3] Previous studies have already reported the presence of different classes of Phytoprostanes in vegetable and olive oils, particularly in linseed and soybean oils, as well as in aqueous pollen extracts.[5, 9, 12] To the best of our knowledge, no reports about the Phytoprostanes content of red wine have been published. Therefore, grape, must, and the vinification procedure, in relation to changes in the Phytoprostanes content of wine or must, is a wide field to explore. It is made even more important by taking into account that the scientific literature has highlighted Phytoprostanes as a representative tool for measuring *in vivo* stress in plants.[4, 6] Phytoprostanes could also be of interest to wineries, in order to know the oxidative status of their products and as a quality control in the winemaking process.

MATERIAL AND METHODS

Standards and reagents

Phytoprostanes are very stable in their frozen form; they can remain unaltered for several years. Phytoprostanes standards (9-F_{1t}-Phytoprostane (Phyto1), *ent*-16-*epi*-16-F_{1t}-Phytoprostane + *ent*-16-F_{1t}-Phytoprostane (Phyto2+3), 9-*epi*-9-D_{1t}-Phytoprostane (Phyto4), 9-D_{1t}-Phytoprostane(Phyto5), 16-B_1-Phytoprostane + *ent*-16-B_1-Phytoprostane (Phyto6+7), 9-L_1-Phytoprostane + *ent*-9-L_1-Phytoprostane (Phyto8+9)) were synthesized according to previous procedures.[13-16]

Two types of solid-phase extraction (SPE) cartridge were used in this study: Chromabond C_{18} columns (100 mg/6mL) were obtained from Macherey-Nagel (Düren, Germany) and Strata X-AW (100 mg/3mL) from Phenomenex (Torrance, CA). Both butylated hydroxyanisole (BHA) and bis-(2-hydroxyethyl)-amino-tris-(hydroxymethyl)-methane (BIS-TRIS) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol (MeOH) was acquired from VWR (Fontenay-sous-Bois, France), acetonitrile was obtained from Merck (Darmstadt, Germany), and *n*-hexane was purchased from Panreac (Barcelona, Spain). All LC-MS grade solvents were obtained from J.T. Baker (Phillipsburg, NJ). Water was treated in a Milli-Q water purification system (Millipore, Bedford, MA).

Red wine samples

Red wines were provided by Baigorri winery (Bodegas Baigorri S.A.U, Samaniego, Álava, Spain). Three different wines were selected in order to study different vinification and aging procedures. For correct maintenance, they were stored between 12 ºC and 14 ºC after bottling.

“Baigorri carbonic maceration 2010” wine (CMW) was made with a combination of tips of bunches from hand-harvested Tempranillo grapes. Before being fermented, the grapes were macerated for a long time. Short

fermentations in stainless-steel tanks were performed. No aging procedure was employed.

"Baigorri aged 2007" wine (AW) was manufactured using the tips of bunches of hand-harvested Tempranillo (90%), Garnacha (5%), and other native grape varieties (5%). Long macerations and intracellular fermentations in stainless-steel tanks were employed. Large oak barrels were employed during the 14 months of aging.

"Baigorri high expression 2010" wine (HEW) was made from hand-harvested Tempranillo grapes selected from very old (more than 50 years) and low yielding vineyards. For its long maceration and fermentation times, stainless-steel tanks were used along the whole process. Large oak barrels were also employed during the 22 months of aging.

The alcoholic grade of CMW and AW was similar (13.5 º) but slightly higher in HEW (14 º).

Must samples

The musts analyzed during the current study were stored at -20 ºC for seven months after the harvest of the grapes, so that the fermentation process did not begin. They were the original grape juices used for the winemaking procedure of each wine. This allows elucidation of the effect of the winemaking process, by direct comparison of each must with its respective wine.

The must samples are referred to in the text as follows: CMM for the initial must of "Baigorri carbonic maceration" wine, AM for the initial must of "Baigorri aged 2010" wine, and HEM for the initial must of "Baigorri high expression 2010" wine.

The chemical composition of the samples was quite similar. Total acidity of must samples was 4.44, 5.81 and 6.58 g/L for CMM, AM and HEM respectively. Finally, density was 1102, 1099 and 1100, while pH was 3.65, 3.42 and 3.52 for CMM, AM and HEM respectively.

Extraction of Phytoprostanes

For the analysis of Phytoprostanes, their extraction from wines and musts was performed following the SPE method developed by Medina et al [17] and Collado-González et al [12], slightly modified for the wine matrix. A mixture of three different bottles of each wine was employed for the extraction. Strata X-AW cartridges were employed for the SPE, and were conditioned with 2 mL of methanol followed by 2 mL of miliQ water. After that, the cartridges were washed with 2 mL of water, 2 mL of methanol/water (1:3,*v:v*), and 2 mL of acetonitrile. Finally, the samples were eluted with 1 mL of methanol. The samples were brought to dryness under vacuum, reconstituted with 200 µL of elution phases A:B (90:10, v:v), and filtered through a Millex HV13 0.45 µm membrane filter (Millipore, Bedford, MA, USA).

UHPLC-QqQ-MS/MS analyses.

Separation of the Phytoprostanes present in the samples was performed using a UHPLC coupled to a 6460 QqQ-MS/MS (Agilent Technologies, Waldbronn, Germany), as previously described.[12] Each sample was analyzed in triplicate. Chromatographic separation was carried out on a BEH 156 2.1 x 50 mm, 1.7 µm C_{18} column (Waters, Milford, MA). The column temperatures were 6 ºC (left side) and 6 ºC (right side). The mobile phases employed were solvent A (water:acetic acid (99.99:0.01, *v:v*)) and solvent B (methanol:acetic acid (99.99:0.01, *v:v*)). The elution was performed at a flow rate of 0.2 mL/min, using

the following gradient profile: 60% B at 0 min, 62% B at 2 min, 62.5% B at 4 min, reaching 65% B at 8 min, and returning to the initial conditions at 8.01 min.

The MS analysis was applied in the multiple reaction monitoring (MRM) negative ESI mode. The ESI conditions and ion optics were as previously described.[12] Data acquisition and processing were performed using MassHunter software, version B.04.00 (Agilent Technologies). The quantitation of Phytoprostanes detected in the wines and musts was performed using authentic standards of 9-F_{1t}-Phytoprostane, 9-*epi*-9-F_{1t}-Phytoprostane, 16-B_1-Phytoprostane, *ent*-16-B_1-Phytoprostane, 9-L_1-Phytoprostane, and *ent*-9-L_1-Phytoprostane. The synthetic isoprostane d_4-15-F_{2t}-IsoP (8-isoPGF$_{2\alpha}$-d_4) was used as the internal standard.

Statistical analysis.

An analysis of variance (ANOVA; Duncan) was applied to establish significant differences between the means obtained for the different samples of wine and must. A probability value of $p<0.05$ was adopted as the criterion for significant differences. These analyses were performed with SPSS version 15 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Qualitative analysis of Phytoprostanes.

The individual Phytoprostanes found in wines and musts are shown in Figure 2. Their identification was confirmed according to their molecular masses, the precursor ions (*m/z* 327.2 and *m/z* 307.2), the characteristic MS/MS fragmentation product ions, and the corresponding retention times. In contrast to prostaglandins, Phytoprostanes are formed non-enzymatically as

183 regio- and stereoisomeric mixtures.[12] The Phytoprostane profiles of the
184 analysed beverage samples indicate the presence in all samples of the 9 and
185 16 series of the F_1- and D_1- classes of Phytoprostanes. The B-series and L-
186 series did not present a well standardized distribution among the different
187 samples. In addition, only one wine (CMW) and one must (HEM) contained
188 enantiomers of the racemic mixtures of 16-B_1 and 9-L_1-Phytoprostane (Phyto
189 6+7 and Phyto8+9). It is important to underline that the analytical conditions
190 employed in this study did not allow separation of the enantiomers of the
191 racemic mixtures of 16-B_1-Phytoprostane + *ent*-16-B_1-Phytoprostane and 9-L_1-
192 Phytoprostane + *ent*-9-L_1-Phytoprostane. Therefore, both enantiomers of L and
193 B series were quantited together.

194 Four Phytoprostanes were identified in all the samples (the three wines and
195 the three musts): Phyto1, Phyto2+3, Phyto4 and Phyto5.

196 **Quantitative analysis of Phytoprostanes**

197 The concentrations of total free Phytoprostanes are represented in
198 Figure 3. This concentration varied widely among the three primary musts. In
199 fact, no significant difference (p>0.05) in the final concentration of total
200 Phytoprostanes was found when comparing the primary must corresponding to
201 carbonic maceration wine (CMM) (48.9±2.6 ng/mL) and the primary must of
202 aged wine (AM) (20.5±0.8 ng/mL). However, the primary must of high
203 expression wine (HEM) had a significantly higher level (p<0.01) of total
204 Phytoprostanes (430.9±15.7 ng/mL) than CMM or AM. As commented on
205 above, HEM came from very old and low yielding vineyards (more than 50
206 years old) which are exposed to more stress factors than newer vineyards.[18]
207 This would probably lead to an increase in pro-oxidant reactive species and to

the subsequent formation of Phytoprostanes by lipid peroxidation of ALA. Consequently, differences in agronomic factors (vineyard age) could have contributed to the different concentrations of total Phytoprostanes, compared to HEM, in the must from grapes grown in the new vineyards (CMM and AM), .

In the three wine samples studied, the total Phytoprostanes concentration did not present a standardized range. Actually, the values did not vary (p>0.05) between aged wine (AW) (213.162±3.06 ng/mL) and high expression wine (HEW) (199.818±4.2 ng/mL), but the total Phytoprostanes concentration of carbonic maceration wine (CMW) (131.747±2.3 ng/mL) was significantly lower (p>0.05).

The levels of individual Phytoprostanes varied consistently among the different classes of Phytoprostanes (Table 1). The F_1-Phytoprostanes series class was generally the most abundant class (p<0.05) found in all the samples, Phyto1 being the most abundant compound (436.6±7.9 ng/mL). Likewise, a change in the proportion of the F_1-Phytoprostane series was observed in both aged wines (AW and HEW). In CMM, AM, HEM, and CMW, Phyto1 was found at a higher concentration (p<0.05) (32.7±1.8 ng/mL; 13.5±0.1 ng/mL; 149.8±1.8 ng/mL and 90±0.9 ng/mL, respectively) than the sum of Phyto2 + Phyto3 (6.8±0.3 ng/mL; 4.5±0.1 ng/mL; 50.1±0.1 ng/mL and 19.9±0.4 ng/mL, respectively). However, in AW and HEW the opposite relationship was found: the sum of Phyto2 + Phyto3 (133.8±2.2 ng/mL and 124.9±1.7 ng/mL, respectively) exceeded the level of Phyto1 (76.95±0.75 ng/mL and 73.57±2.4 ng/mL, respectively). Therefore, transformations of the stereoisomers during the aging of wines could change the proportion of the F_1-Phytoprostanes series.

The D_1-Phytoprostanes series class was found primarily in HEM. Two epimeric compounds (Phyto4 (25.1±2.1 ng/mL) and Phyto5 (200.4±11.1 ng/mL)) were abundant in HEM. However, the level of Phyto4 in the rest of the samples did not exceed 0.6 ng/mL, whereas Phyto5 was also plentiful in CMW (17.09±0.7 ng/mL). Minor amounts were found in CMM and AM (8.7±0.4 and 2.3±0.5 ng/mL, respectively), with even lower values in the rest of the samples.

As commented above, the analytical conditions employed in the present study did not allow the separation of the different enantiomers. Therefore, enantiomers of both the 9-L and 16-B-Phytoprostanes series of B_1- and L_1-Phytoprostanes were quantitated together. Consequently, these two classes were identified and quantitated as the sum of 16-B_1 + 9-L_1-*ent*-16-B_1-Phytoprostane and the sum of 9-L_1 + *ent*-16-$B_1$9-L_1-Phytoprostane, respectively. Finally, compounds from the B_1 and L_1-Phytoprostanes classes (Phyto6+7 and Phyto8+9) were found in very low amounts. Only in CMW and HEM were they abundant enough to be quantitated, according to the limit of quantitation of the method developed by Collado-González et al.[12] In fact, in these two samples, the sums of the concentrations of Phyto6+7 (2.8±0.1 ng/mL) and Phyto8+9 (10.3±0.5 ng/mL) were not significant, compared to the quantities found in the other Phytoprostanes classes (p<0.05).

The vinification process seems to modify the initial content of Phytoprostanes in the must, since wines CMW and AW showed higher total Phytoprostanes concentrations than CMM and AM (their corresponding primary musts). The musts CMM (48.7±2.4 ng/mL) and AM (20.4±0.7 ng/mL) had lower concentrations (p<0.05) of total Phytoprostanes than their respective finished wines (131.8±2.1 ng/mL and 213±2.93 ng/mL for CMW and AW, respectively).

The wine aging process may be an important factor in the formation of Phytoprostanes. The musts CMM and AM exhibited similar (p>0.05) Phytoprostanes levels. Nevertheless, after the vinification process, the total Phytoprostanes concentration in CMW was lower than in AW. In the case of CMW, this concentration could have been a consequence of the carbonic maceration process (extraction) applied to the grapes and the consequent alcoholic and malolactic fermentation in stainless-steel tanks.[19] However, the vinification of AW could have been more oxidative, owing to the use of oak-wood barrels for the aging procedure employed in the vinification. This could have released pro-oxidant compounds into the medium, in addition to promoting greater contact with oxygen.[20] In wine, ROS can be produced by reduced transition metals ions like copper or iron. Superoxide radical anions, hydroperoxyl radicals, or hydrogen peroxide could be responsible for the oxidation of ALA.[21] The vinification of AW included an aging process that was not included in the production of CMW. The oxidation processes during this vinification, besides the aging of the red wine, could have led to the production of reactive pro-oxidant molecules which oxidize ALA to Phytoprostanes, explaining the difference in the final level of total Phytoprostanes between CMW and AW.

By contrast, in the vinification process that yielded HEW from HEM, the primary must showed a total Phytoprostanes concentration that was more than two-fold higher (430.9±15.7 ng/mL) than that of the finished wine (199.8±4.2 ng/mL). This reduction could be mainly attributable to the great loss of the 9 series of the D_1-Phytoprostanes class. It is important to highlight that the D_1-Phytoprostane class is the only studied that is not a terminal compound (end

products) in the non-enzymatic lipid peroxidation. In this sense, the decline of the total Phytoprostanes content may be explained by the rearrangement into J_1 and dJ_1-Phytoprostanes, by a dehydration reaction, of the Phyto6, present in large amounts in HEM (200.424±11.192 ng/mL). This probably occurred along the vinification process of HEW. Since pro-oxidant compounds are present during the vinification process, the stability of intermediate Phytoprostanes is uncertain.[3, 22] The oxidizing conditions during the vinification process probably led to the oxidation of ALA, and thus the formation of D_1-Phytoprostanes. The D_1-Phytoprostanes may have been oxidized to J_1 and dJ_1-Phytoprostanes, but this could not be investigated, as there are no authentic markers available for these compounds).

To our knowledge, no reports which describe the Phytoprostanes content in wine or must have been published. Nevertheless, a few plant matrices have been investigated in order to report the Phytoprostanes occurrence.[3] Tobacco, *Arabidopsis thaliana, Crotalaria cobalticola, Eschscholzia californica*, and birch pollen have shown the presence of Phytoprostanes. Birch pollen had the highest Phytoprostanes concentration of all these matrices (32 μg/g).[23] Consequently, the selection of the plant tissue analyzed is very important, since ROS are not produced equally throughout all the structures of the plant. In fact, green tissues are the most likely producers of ROS, because of the singlet oxygen formed in the chloroplast during photosynthesis, leading to an increase in the number of peroxidation products.[22] Savchenko et al [24] reported that the total amount of Phytoprostanes in photosynthetic tissue is ten times higher than in roots. In this sense, depending on the tissue studied, Phytoprostanes levels might vary extensively. Few researchers have reported Phytoprostanes

contents in foods. Durand et al [22] described high amounts of Phytoprostanes in tomato leaves, F_1, E_1, and d-J_1-Phytoprostanes being the most abundant. The level of Phytoprostanes in almonds and olive/sunflower oil has been reported to range from 4.0 to 23.8 ng/100g.[12, 25, 26]

Researchers have highlighted the importance of Phytoprostanes as bioactive lipid derivatives, not only in plant matrices, but also in mammalian systems. [27] Phytoprostanes, derived from a non-enzymatic oxidation reaction, are believed to exert beneficial effects in the organism.[3, 5, 27] Due to their similarities to different prostaglandins, they could mimic the effects of the latter on the organism.[4, 22, 28] In the present survey, F_1-Phytoprostanes were the most abundant class of Phytoprostanes in all the samples studied (436.6±7.9 ng/mL for 9-F_{1t}-Phytoprostane and 340.1±4.8 ng/mL for e*nt*-16-*epi*-16-F_{1t}-Phytoprostane). These Phytoprostanes can regulate inflammatory responses in dendritic cells.[23] Karg et al [5] reported that A_1 and B_1-Phytoprostanes inhibited the release of nitric oxide in lipopolysaccharide-stimulated RAW264.7 macrophages. Thus, cardiovascular diseases could be ameliorated by the effects of Phytoprostanes. In fact, Barden et al [28] related the intake of F_1-Phytoprostanes to protective effects on the cardiovascular system.

The importance of the intake of Phytoprostanes could be related to neuroprotective effects too. Minghetti et al [27] showed that B_1-Phytoprostanes were biologically active in experimental models of immature cells of the central nervous system, exhibiting neuroprotective effects against oxidant injury induced by hydrogen peroxide and promoting myelination through mechanisms which involve activation of the peroxisome proliferator-activated receptor (PPAR)-γ.

Bioavailability of Phytoprostanes has also been demonstrated *in vivo* with healthy humans. Barden et al [28] examined the effect of flaxseed oil, containing arachidonic acid; they examined the effect of a diet supplemented with flaxseed oil on F_1-Phytoprostanes and F_2-Isoprostanes concentrations in the urine and plasma of healthy men. Both the plasma and urine analyses confirmed the absorption of Phytoprostanes by the intestinal tract. The esterified and non-esterified Phytoprostanes levels before intake of flaxseed oil were higher in plasma than in urine. Not only oil has been demonstrated to contain Phytoprostanes; parenteral nutrition [5] has also shown a significant content of these metabolites (0.09-99 mg/L).

Assuming these possible beneficial effects, Phytoprostanes would have an important impact on the Mediterranean diet, due to the wide consumption of wine around the world. The F_1-Phytoprostanes concentrations found in the red wines and musts, and the suggested beneficial effects on the organism, make their contribution relevant in the beneficial effects of the Mediterranean diet. However, further studies seem to be necessary to understand the physiological relevance of Phytoprostanes in general and of F_1-Phytoprostanes in particular; for example, their role in preventing myocardial infarction or heart illness.

To the best of our knowledge, this is the first report describing the presence of Phytoprostanes in wine or must. The results showed the F_1-Phytoprostanes as the most abundant class for all samples. Likewise, D_1-Phytoprostanes were present in musts in large quantities, especially in HEM. Vinification and aging procedures may influence and change the initial Phytoprostane profile, favoring the formation of pro-oxidant species. Further

studies are needed to elucidate the development of Phytoprostanes during wine production.

Taking into account the possible beneficial effects of Phytoprostanes in the cardiovascular system and the high concentrations observed in wines and musts, Phytoprostanes could be an important factor in the cardioprotective or cerebrovascular effects of red wine and the Mediterranean diet, due to their possible anti-inflammatory effects. However, further clinical trials with humans and with animal models are necessary to elucidate how Phytoprostanes could improve the cardiovascular system or exert neuroprotective effects.

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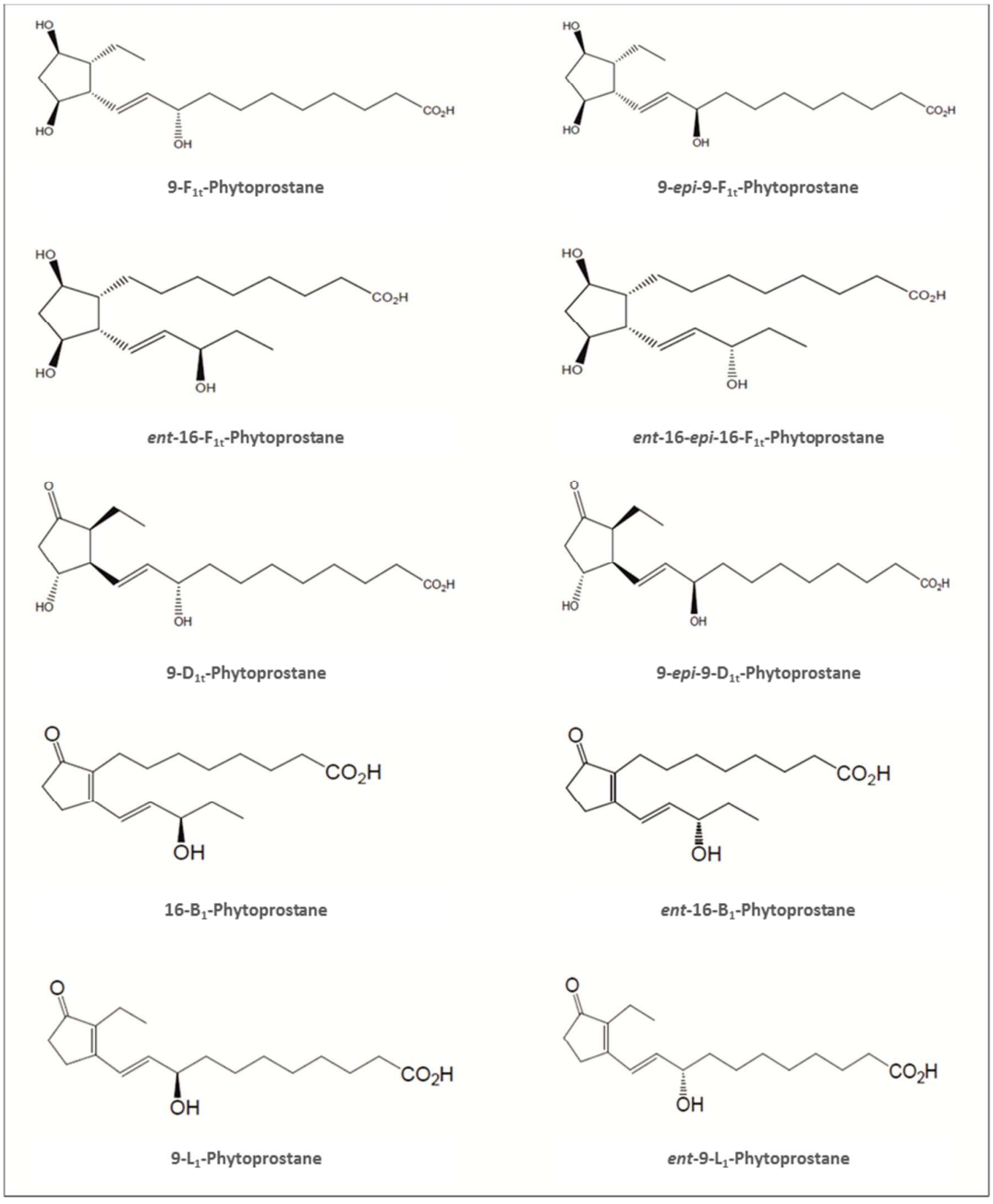
467

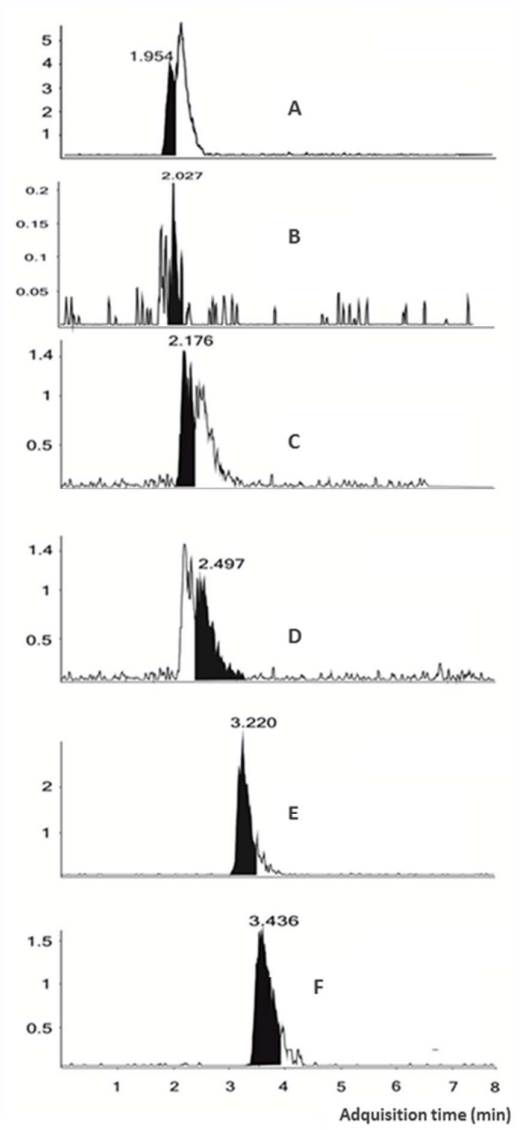
FIGURE CAPTIONS

Figure 1. Metabolites of the different families of Phytoprostanes.

Figure 2. Phytoprostanes chromatograms for CMW, measured by UPLC-MS/MS. A=9-F_{1t}-Phytoprostane, B=*ent*-16-*epi*-16-F_{1t}-Phytoprostane + *ent*-16-F1t-Phytoprostane, C=9-*epi*-9-D_{1t}-Phytoprostane, D=9-D_{1t}-Phytoprostane, E=16-B_1-Phytoprostane + *ent*-16-B1-Phytoprostane, F=9-L_1-Phytoprostane + *ent*-9-L1-Phytoprostane.

Figure 3. Concentrations of total Phytoprostanes. CMW/CMM: wine/must with carbonic maceration; AW/AM: aged wine/must; HEW/HEM: high expression wine/must.


9-F_{1t}-Phytoprostane
9-*epi*-9-F_{1t}-Phytoprostane
ent-16-F_{1t}-Phytoprostane
ent-16-*epi*-16-F_{1t}-Phytoprostane
9-D_{1t}-Phytoprostane
9-*epi*-9-D_{1t}-Phytoprostane
16-B_1-Phytoprostane
ent-16-B_1-Phytoprostane
9-L_1-Phytoprostane
ent-9-L_1-Phytoprostane


1.954
A
2.027
B
2.176
C
2.497
D
3.220
E
3.436
F
Adquisition time (min)

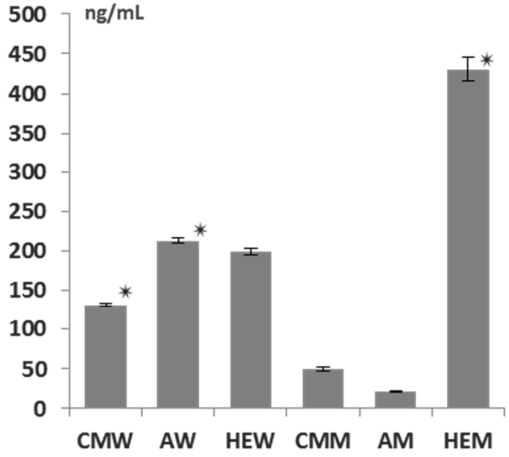
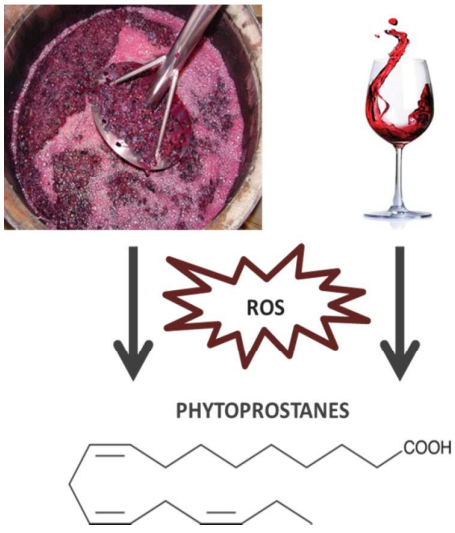

ng/mL
500
450
400
350
300
250
200
150
100
50
0
CMW
AW
HEW
CMM
AM
HEM

Table 1. Content of Individual Phytoprostanes Analyzed in Three Types of Musts and Wines

ESTRUCTURE NUMBER	PHYTOPROSTANES	CMW	AW	HEW	CMM	AM	HEM
(Phyto1)	9-F_{1t}-Phytoprostane	90.04±0.9	76.9±0.7	73.5±2.4	32.7±1.8	13.5±0.1	149.8±1.8
(Phyto2+3)	*ent*-16-*epi*-16-F_{1t}-Phytoprostane + *ent*-16-F_{1t}-Phytoprostane	19.9±0.3	133.8±2.2	124.9±1.7	6.7±0.2	4.5±0.08	50.1±0.08
(Phyto4)	9-*epi*-9-D_{1t}-Phytoprostane	0.4±0.02	0.1±0.02	0.09±0.005	0.6±0.03	0.1±0.06	21.5±2.1
(Phyto6)	9-D_{1t}-Phytoprostane	17.09±0.7	2.2±0.01	1.2 ±0.01	8.7±0.4	2.3±0.5	200.4±11.1
(Phyto6+7)	16-B_1-Phytoprostane + *ent*-16-B_1-Phytoprostane	0.3±0.001	ND	ND	ND	ND	2.5±0.1
(Phyto8+9)	9-L_1-Phytoprostane + *ent*-9-L_1-Phytoprostane	3.8±0.2	ND	ND	ND	ND	6.4±0.3

ND: Not Detected. CMW/CMM: Carbonic Maceration Wine/Must; AW/AM: Aged Wine/Must; HEW/HEM: High Expression Wine/Must. Results Are Expressed in ng/mL±SD.

TABLE OF CONTENTS GRAPHIC



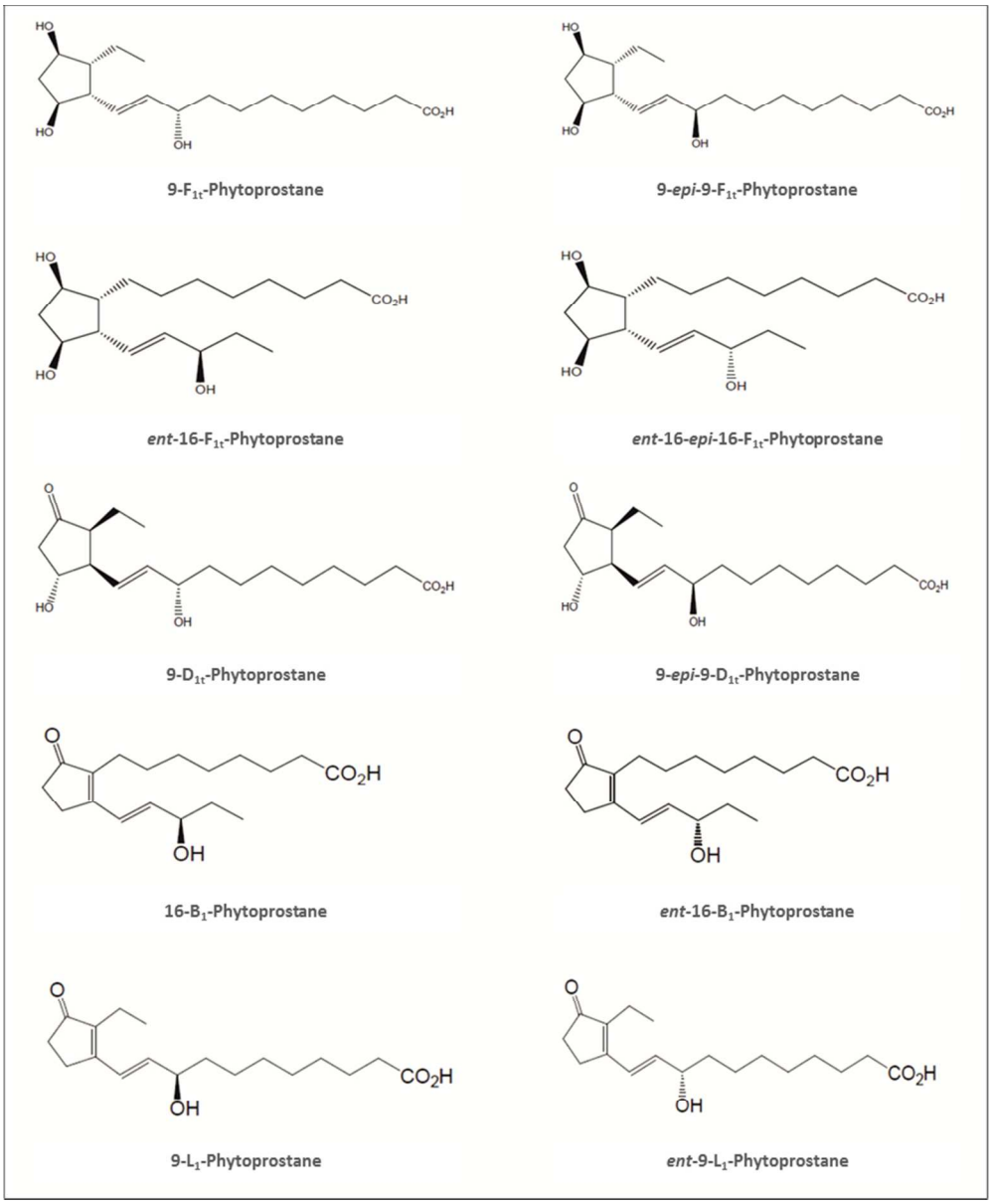


Figure 1. Metabolites of the different families of Phytoprostanes.
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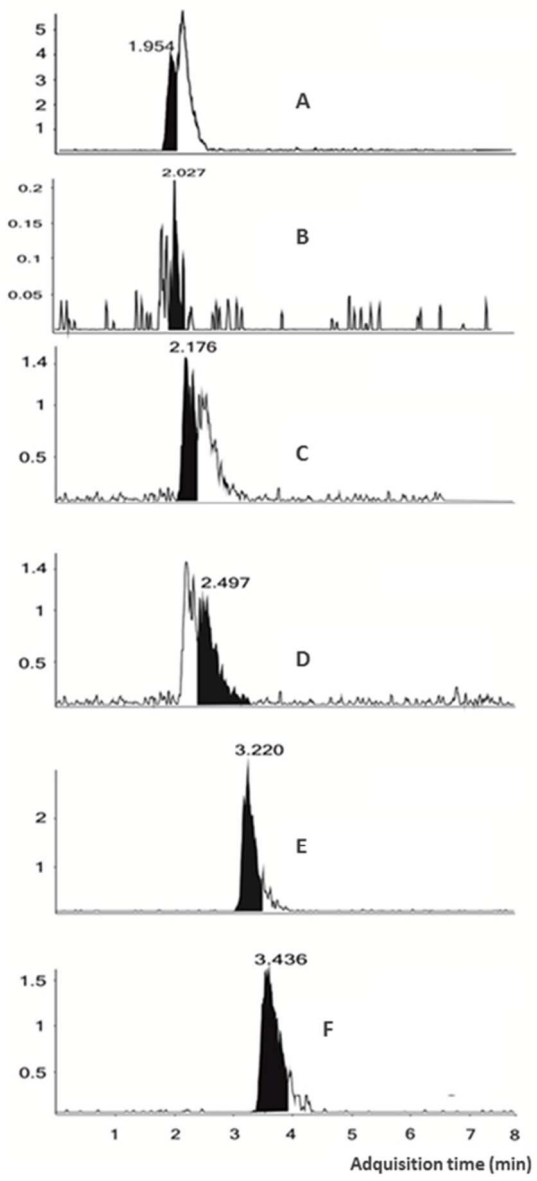

Figure 2. Phytoprostanes chromatograms for CMW, measured by UPLC-MS/MS. A=9-F1t-Phytoprostane, B=ent-16-epi-16-F1t-Phytoprostane + ent-16-F1t-Phytoprostane, C=9-epi-9-D1t-Phytoprostane, D=9-D1t-Phytoprostane, E=16-B1-Phytoprostane + ent-16-B1-Phytoprostane, F=9-L1-Phytoprostane + ent-9-L1-Phytoprostane.
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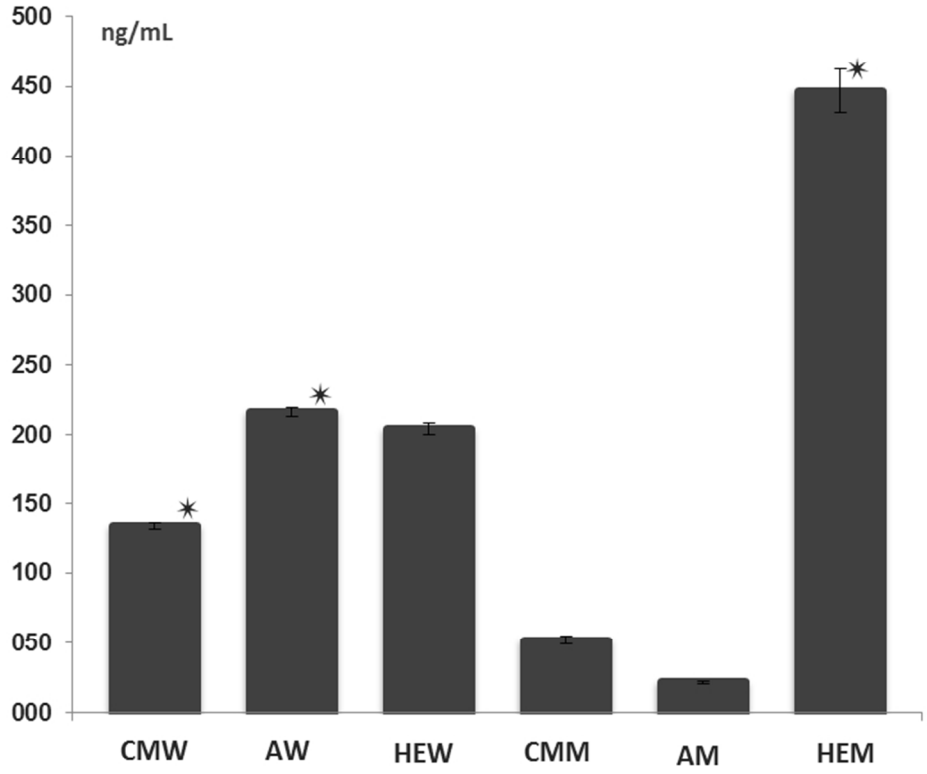

Figure 3. Concentrations of total Phytoprostanes. CMW/CMM: wine/must with carbonic maceration; AW/AM: aged wine/must; HEW/HEM: high expression wine/must.
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TOC Graphic
150x41mm (150 x 150 DPI)