Winery by-products as a valuable source for natural antihypertensive agents

Raúl López-Fernández-Sobrino†, Cristina Torres-Fuentes†, Francisca Isabel Bravo* and Begoña Muguerza

Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Tarragona, Spain; raul.lopez@urv.cat (R.L-F-S); cristina.torres@urv.cat (C.T-F); begona.muguerza@urv.cat (B.M).

*Corresponding author: franciscaisabel.bravo@urv.cat; Tel.: +34-977-558-837

† These authors contributed equally to this work.

Winery by-products as a valuable source for natural antihypertensive agents

Hypertension (HTN) is one of the leading causes of death in the world. Agri-food by-products are emerging as a novel source of natural antihypertensive agents allowing for their valorization and making food and agricultural industries more environmentally friendly. In this regard, wine making process generates large amounts of by-products rich in phenolic compounds that have shown potential to exert several beneficial effects including antihypertensive properties. The aim of this study was to review the blood pressure-lowering effects of winery by-products. In addition, molecular mechanisms involved in their bioactivity were also evaluated. Among the winery by-products, grape seed extracts have widely shown antihypertensive properties in both animal and human studies. Moreover, recent evidence suggests that grape stem, skin and pomace and wine lees may also have great potential to manage HTN, although more studies are needed in order to confirm their potential in humans. Improvement of endothelial dysfunction and reduction of oxidative stress associated with HTN are the main mechanisms involved in the blood pressure-lowering effects of these by-products.

Keywords: antioxidant activity, blood pressure, grape, hypertension, phenolic compound, wine

Introduction

Hypertension (HTN), defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, is a common risk factor for cardiovascular disease (CVD) and a major global public health challenge (Unger et al. 2020). In the last years, there have been an increase in HTN prevalence attributed to unhealthy diets (i.e., high sodium and low potassium intake) and lack of physical activity. According to the World Health Organization, 1.28 billion of people worldwide suffer HTN and only 21 % are under control (World Health Organization 2021). HTN is also associated with other diseases. Thus, more than 50 % of hypertensive people have other common cardiovascular risk factors such as diabetes, lipid disorders, overweight, obesity or metabolic syndrome (Unger et al. 2020). Antihypertensive therapy has been shown to reduce the risk of CVD. Hence, it has been suggested that the reduction of both DBP and/or SBP by only 5 mmHg and 10 mmHg respectively, reduce the risk of suffering cardiovascular events (Thomopoulos et al. 2014). Therefore, treatments and prevention strategies for lowering blood pressure (BP) and slowing the progression of HTN are crucial.

In patients with HTN or a pre-existing cardiovascular risk of 10 % or higher, both lifestyle changes and medication are recommended (Muntner and Whelton 2017). Current available treatments include five classes of drugs according to their targets: angiotensin-converting enzyme (ACE) inhibitors, angiotensin (Ang) II receptor blockers, beta-blockers, diuretics and calcium-channel blockers (Mancia and Zanchetti 2008). Among these, ACE inhibitors are the first-line HTN treatment and act on the renin-angiotensin-aldosterone system (RAAS) inhibiting the key enzyme in the control of BP. Thus, ACE catalyzes the conversion of Ang I to Ang II, which binds to Ang II type-1 receptor (AT1R) causing vasoconstriction. However, these treatments may present side effects such as hypotension, dizziness, hyperkalaemia or increase creatinine levels among others (Margalef et al. 2017). Moreover, these drugs are not suitable for treating pre-hypertensive individuals at risk of CVD as they may have different metabolic side effects. Thus, implementation of lifestyle modifications is the most recurrent method in these patients (Collier and Landram 2012). However, it is important to act at this pre-hypertensive stage with the aim to avoid the

development of HTN (Margalef et al. 2017). Therefore, there is a need to develop new therapeutic antihypertensive treatments with reduced side effects and suitable for pre-hypertensive subjects.

According to the Food and Agriculture Organization of the United Nations, about 1.3 billion tonnes of foods are lost or wasted globally (Food and Agriculture Organization of the United Nations 2020). However, these food by-products are of high interest for both scientific and food industry communities as they are a great source of bioactive compounds, mainly phenolic compounds and bioactive peptides, with several beneficial effects including antihypertensive (Margalef et al. 2017). Indeed, some of these compounds have been found to exert ACE inhibition properties (Sturrock et al. 2019). Therefore, the use of these agri-food by-products as a novel source of natural bioactive compounds contribute to more environmentally friendly food and agricultural industries (Del Borghi et al. 2020).

Grapes are one of the world's largest fruit crops and 57% of its production is destined to make wine generating large amounts of by-products (International Organisation of Vine and Wine 2019). Grapes, wine and some winery by-products such as grape seeds, have shown different beneficial effects on BP attributed to their content in bioactive compounds, mainly phenolic compounds (Dohadwala and Vita 2009). Therefore, the objective of this review was to discuss the potential of winery by-products as sources of antihypertensive phenolic compounds. In addition, the mechanisms involved in the BP-lowering effect of these by-products are discussed.

Antihypertensive effect of grape phenolic compounds

Grape phenolic compounds have widely shown to exert several beneficial effects including antihypertensive properties. Different mechanisms have been reported to be involved in their antihypertensive effects, including ACE inhibitory activity. As mentioned above, this enzyme is key in BP regulation as it produces the vasoconstrictor Ang II. Ang II also induces the production of aldosterone in the adrenal glands, leading to the retention of Na^+ and water and increasing BP (Cachofeiro et al. 2008). Therefore, its inhibition is one of the main targets when looking for compounds with BP-lowering effects.

ACE inhibitory activity of flavonoids have been related with specific sub-structures on the flavonoid skeleton, mainly the catechol group in the B-ring, the double bond between the C2 and C3 positions in the C-ring, and the ketone group at the C4 postion in the C-ring (Guerrero et al. 2012). Furthermore, administration of individual flavanols such as catechin and epicatechin, which are abundant in different grape by-products, also led to BP reduction in hypertensive animals (Quiñones et al. 2015). These beneficial effects have been linked to increased nitric oxide (NO) production, the main endothelium-derived vasodilator factor, in endothelial cells. Their antihypertensive properties were also related to the strong antioxidant potential of phenolic compounds. Thus, overproduction of reactive oxygen species (ROS) contributes to the development of CVD and endothelial dysfunction (Cheng et al. 2017). In blood vessels, high levels of ROS are related with low levels of vasodilators, generating endothelial dysfunction. NO is the main vasodilator affected as it interacts with oxygen free radicals to generate peroxynitrite. This leads to both reduced NO availability and increased ROS levels promoting HTN development (Rodrigo et al. 2015). Furthermore, the increment of ROS levels in cardiovascular control organs elicit targeted immune response, which potentiates systemic HTN and its complications. Thus, oxidative stress induced by pro-inflammatory signals worsens the immunologic response in endothelium, which produces the progressive deterioration of vascular function (Crowley 2014). Moreover, high levels of oxidative stress in hypertensive states increase Ang II levels, which stimulate the production of ROS through NADPH oxidase (NOX) (Masaki and Sawamura 2006). Isoforms of this enzyme, specifically NOX1/NOX2, are involved in the development of endothelial dysfunction, HTN and inflammation (Konior et al. 2014). Therefore, antioxidant properties of polyphenols can also protect from increased ROS levels contributing to vascular homeostasis.

In addition to flavanols, red grapes are also rich in anthocyanins, which have also been studied for their health-promoting effects, including vascular benefits (Rodriguez-Mateos et al. 2019). In this regard, the anthocyanin malvidin-3-glucoside has been reported as a potent vasodilator (Calfío and Huidobro-Toro 2019). Anthocyanins have also demonstrated *in vitro* antioxidant properties, preventing oxidative damage,

increasing NO bioavailability and reducing NOX activity in lipopolisacharide-stimulated RAW 264.7 cells (Kim et al. 2017). In addition, intake of anthocyanin-rich extracts from different sources led to increased endothelial NO levels via the regulation of endothelial NO synthase (eNOS) expression and activity, promoting vasorelaxation (Bell and Gochenaur 2006). Moreover, different meta-analyses of randomized controlled trials demonstrated an association of anthocyanins consumption with significant reduced SBP and improved vascular function. Thus, a meta-analysis involving 24 clinical studies in healthy and hypertensive subjects showed that acute and chronic anthocyanin consumption improved flow-mediated dilatation promoting vascular health. Acute administration also led to reduced pulse (Fairlie-Jones et al. 2017). In addition, a meta-analysis conducted with 22 clinical trials in healthy and hypertensive subjects showed that consumption of berries rich in anthocyanins significantly reduced CVD risk factors including SBP, low density lipoprotein-cholesterol and fasting glucose levels (Huang et al. 2016). Recently, hydroalcoholic extracts of Patagonian Calafate berry (*Berberis microphylla*) containing conjugated anthocyanins, mainly glycosylated anthocyanidins such as delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside, showed strong vasodilatation effects in a rat arterial mesenteric bed bioassay (Calfío and Huidobro-Toro 2019). Vascular responses of these glycosylated anthocyanins were endothelium-dependent, mediated by NO production and independent of antioxidant capacity.

Among the stilbenes family, resveratrol is the most abundant in grapes and red wine (Bonnefont-Rousselot 2016). Resveratrol has shown antihypertensive properties in different models of hypertensive rats associated with the improvement of endothelium through vascular relaxation, enhanced eNOS activity, subsequently increasing NO (Mizutani et al. 2000; Miatello et al. 2005; Bhatt et al. 2011; Cheng et al. 2020). Its antihypertensive potential has also been related to its antioxidant capability, increasing superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and reduced glutathione (GSH) activities (Bhatt et al. 2011; Alturfan et al. 2012; Bagul et al. 2012; Ramar et al. 2012; Franco et al. 2013; Xia et al. 2017; Cheng et al. 2020). Furthermore, resveratrol activates SIRT such as SIRT1, which is implicated in the deacetylation and consequent activation of eNOS (Gertz et al. 2012). However, there is

some controversy regarding the effects of resveratrol on BP in humans. Nevertheless, the potential use of this phenolic compound as antihypertensive agent has been recently pointed out, mostly when administered at high daily dose (≥300 mg/day) and in diabetic patients (Fogacci et al. 2019).

Rutin, quercetin and kaempferol are the main phenolic compounds regarding the flavonol group in grapes (Iglesias-Carres et al. 2018). Quercetin stands out for its capacity of improving endothelial function by increasing NO levels, reducing the endothelial vasoconstrictor factor endothelin-1 (ET-1), and preventing apoptosis in endothelial cells (Marunaka et al. 2017). Moreover, quercetin modulates enzymes with radical scavenging ability such as hemeoxygenase-1 (HO-1) and reduces ROS production in macrophages (Luo et al. 2019). In hypertensive animals, quercetin presented the ability to inhibit ACE, reducing BP levels (Larson et al. 2012). Moreover, quercetin decreased SBP (Egert et al. 2009) and both SBP and DBP (Marunaka et al. 2017) in patients with metabolic syndrome and hypertension respectively. Endothelial function was also improved in healthy men as shown by a randomized, placebo-controlled, crossover trial where the administration of quercetin increased the levels of NO and reduced the levels of the vasoconstrictor ET-1 (Loke et al. 2008). Moreover, a randomised double-blinded controlled cross-over trial showed that intake of a quercetin-rich onion skin extract reduced the arterial BP probably by the regulation of ET-1 production in pre-hypertensive subjects (Brüll et al. 2015). In addition, a meta-analysis of 7 randomized controlled trials concluded that supplementation with quercetin reduced both SBP and DBP (Serban et al. 2016).

Gallic acid (GA) is the main phenolic acid found in grapes. The intake of 320 mg GA/kg bw for 16 weeks reduced both SBP and left ventricular hypertrophy in spontaneously hypertensive rats (SHR), which is a major risk factor for cardiovascular diseases (Jin et al. 2017). In this same study, GA attenuated GATA-mediated NOX2 promoter activation in H9c2 cells and inhibited cardiac *Nox2* expression in SHR. Moreover, GA isolated from *Spirogyra sp*. exerted a vasorelaxant effect mediated by the production of NO in human umbilical vein endothelial cells (HUVECs) and an antihypertensive effect in SHR mediated by its vasorelaxant effect, improving the endothelial dysfunction (Kang et al. 2015).

According to the antihypertensive activity exhibited by grape phenolic compounds, the consumption of whole grapes and wine have also shown BP-lowering properties in both animal models and humans (**Tables 1 and 2**). In this regard, the administration of grape powder (600 mg/day) to SHR produced an improvement of the endothelial function mediated by increased eNOS production, and reduced BP (10 mmHg) (Thandapilly et al. 2012). Moreover, administration of 330 mg of grape powder to Sprague-Dawley rats with buthionine sulfoximine-induced HTN reduced BP by 20% via extracellular signal-regulated protein kinase-1/2 (Allam et al. 2013). Regarding wine, most of their beneficial effects have been mostly attributed to its content in resveratrol. However, as discussed above, other phenolic compounds found in wine such as anthocyanins and flavanols have also shown beneficial properties (Snopek et al. 2018). Administration of dried red wine polyphenols at 40 mg/kg/day during four weeks produced a reduction of SBP by 18% and increased *eNos* expression and NO levels in Nw-nitro-L-arginine-methyl-ester (L-NAME)-induced hypertensive rats (Pechánová et al. 2004). In addition, the intake of 150 mg/kg/day of dried red wine polyphenols for 21 days prevented Ang II-induced expression of *Nox subunits 1* and *p22phox* in rat aorta and vascular ROS production. This protective effect is probably due to the ability of red wine polyphenols to reduce vascular oxidative stress and to induce an unaltered endothelial formation of NO in pathologic arteries (Sarr et al. 2006). An increase of NO levels was also observed in HUVECs and HUVEC-derived cell line EA.hy926 treated with an alcohol-free red wine phenolic extract. Moreover, this wine phenolic extract increased eNOS levels and promotor activity (Leikert et al. 2002). Therefore, the enhancement of NO production seems to be the main mechanism involved in the antihypertensive effects of red wine phenolic compounds.

In addition, different clinical studies have evaluated the antihypertensive effect of grape phenolic compounds obtained from different sources. Controversial effects were observed as BP was reduced in some studies (Jiménez et al. 2008; Sivaprakasapillai et al. 2009; Barona et al. 2012; Queipo-Ortuño et al. 2012) while it did not change in others (Ward et al. 2005; Sano et al. 2007; Mellen et al. 2010). This can be due to differences in dosage, time of administration, phenolic compound source (wine, grape seed extracts

and grape polyphenol powder preparations) and participant conditions. However, a meta-analysis with 10 randomized controlled trials in patients suffering different diseases (HTN, metabolic syndrome, coronary disease or at high vascular risk), concluded that grape phenolic compound consumption for at least two weeks, could significantly decrease SBP by 1.48 mmHg but did not affect DBP compared to healthy subjects (Li et al. 2015). Moreover, this study also revealed that the BP-lowering effect of grape phenolic compounds was better when volunteers consumed low-doses of these compounds (< 733 mg/day). In addition, several studies have shown cardioprotective effects, including BP-lowering effects and improvement of endothelial function, as well as antioxidant properties after a moderate consumption of red wine (Chiva-Blanch et al. 2012; Queipo-Ortuño et al. 2012). Moreover, differential effects of red wine have been reported when alcohol is removed. In this regard, a clinical trial carried out in hypertensive subjects showed that the moderate consumption of red wine (272 mL/day for 4 weeks) slightly reduced SBP (2.3 mmHg) and DBP (1 mmHg). Nevertheless, the effect was higher when volunteers consumed the same red wine without alcohol, showing a reduction of 5.8 and 2.3 mmHg in SBP and DBP, respectively (Chiva-Blanch et al. 2012). Moreover, an increase of plasmatic NO levels after dried wine consumption was also observed in these subjects. Furthermore, vasoconstrictor ET-1 plasmatic levels were reduced after 2 hours of either wine or dealcoholized wine consumption in healthy volunteers (Kiviniemi et al. 2010).

Antihypertensive effect of winery by-products

Winery by-products can be used to obtain phenolic-enriched extracts with a wide range of functionalities (Shrikhande 2000). In this regard, several studies in both animals and humans have demonstrated antihypertensive effects of winery by-products and derived extracts (**Table 1 and 2**). In this regard, a recent meta-analysis compromising 28 studies and a total of 1344 participants revealed that grape products including extracts from winery by-products can significantly reduce both SBP and DBP (Asbaghi et al. 2021a). Moreover, **Figure 1** shows the main molecular mechanisms involved.

Grape pomace

In vitro studies have demonstrated that dry grape pomace extract exerts antioxidant effects in endothelial and muscle cells through the increment of gamma-glutamylcysteine synthetase (GCS) and glutathione S-transferase (GST) enzymes (Goutzourelas et al. 2015). GCS is the first enzyme in the biosynthetic pathway of glutathione (GSH), with a critical role for cell survival. GSH protects the cells from oxidative damage, being the most important antioxidant in cells (Dalton et al. 2004). GST is induced under oxidative stress conditions and is involved in the detoxification of organic epoxides, hydroperoxides and unsaturated aldehydes formed after lipid peroxidation (Forman et al. 2009). GST detoxifies these products through their conjugation with GSH. Moreover, administration of a seedless red wine pomace seasoning to SHR at a dose of 300 mg/kg/day for 4 weeks, reduced BP and oxidative damage (Del Pino-García et al. 2017). Restoration of *eNos*, mitochondrial superoxide dismutase (*Sod2*) and *Ho-1* gene expression, reduction of *Ace* aortic gene expression and aorta ROS production and increase of aorta eNOS phosphorylated levels were the leading mechanisms involved in the reduction of BP mediated by the seedless grape pomace (Del Pino-García et al. 2017; Gerardi et al. 2020a). Moreover, this extract also reduced aortic thickness of SHR (Gerardi et al. 2020b). In addition of evidence from *in vitro* and animal studies, the antihypertensive effect of a grape pomace-derived seasoning has been recently reported in volunteers with high pressure levels (>130 mmHg SBP and >85 mmHg DBP) consuming this product (2g /day, 6 weeks). Specifically, a significant decrease in SBP, DBP and mean of BP (16.75 %) was observed respect to the non-treated group (Taladrid et al. 2022a). A reduction of SBP and DBP (4.3 and 5.3 mmHg, respectively) was also observed in subjects with metabolic syndrome consuming 20 g/day of grape pomace flour for 16 weeks (Urquiaga et al. 2015).

Grape seeds

Grape seeds are the most studied grape by-products regarding their antihypertensive effects which have been attributed to their phenolic compounds content, especially proanthocyanins (Gupta et al. 2020). In this regard, antihypertensive mechanisms of a grape seed proanthocyanidin extract (GSPE) have been widely

reported in many *in vitro* and *in vivo* (animal and human) studies. Thus, acute administration of different doses of GSPE (250, 375 and 500 mg/kg bw) reduced SBP and DBP in both SHR (Quiñones et al. 2013) and cafeteria diet-induced hypertensive rats (CHR) (Pons et al. 2016a). The intermediate dose of 375 mg/kg bw was the most effective and also ameliorated oxidative stress (Pons et al. 2016a). These results were in line with other studies in SHR models treated with GSPE (Quiñones et al. 2013) or other extracts rich in flavanol-rich compounds such as cocoa (Cienfuegos-Jovellanos et al. 2009) or cocoa extract (Quiñones et al. 2011), which showed that higher doses are less effective compared to intermediate doses. These findings could be explained by the pro-oxidant properties and the excessive production of ROS caused by high doses of flavanols (López-Fernández-Sobrino et al. 2021d). In addition, no effects were observed in normotensive rats after acute GSPE consumption. Regarding chronic administration, preventive administration of lower doses of GSPE, including 25, 100 and 200 mg/kg bw, were enough to attenuate BP and improve lipid profile in CHR (Pons et al. 2017). Dose of 200 mg/kg bw also reduced body weight and waist perimeter. In addition, chronic corrective administration of GSPE (25 mg/kg bw/day for three weeks) to CHR reduced SBP and DBP by 15 and 10 mmHg, respectively (Mas-Capdevila et al. 2020). Other grape seed extracts obtained from Grenache or Syrah grape varieties have shown little effects on SBP after being administered to SHR (mg/kg/day) for 6 weeks (2 weeks of treatment+1 week of treatment interruption+2 weeks of treatments). However, authors did not discard the potential effect of these two extracts against HTN since it was observed a "rebound effect" (SBP increase in the week without treatment), which is a usual phenomenon in anti-hypertensive drugs (Rasines-Perea et al. 2018).

Increased NO availability and, at a less extent, the vasodilator prostacyclin I2 (PGI2) pathway, have been shown to be involved in these GSPE antihypertensive effects (Quiñones et al. 2014). In addition, the involvement of SIRT1 and ET-1 in the BP-lowering effect of GSPE has also been observed in both SHR and CHR (Pons et al. 2016b). Agreeing to this, administration of GSPE (250 mg/kg/day for five weeks) to ouabain-induced hypertensive Sprague-Dawley (SD) rats was shown to decrease BP by regulating NO and ET-1 balance and suppressing the transforming growth factor-β1 (TGF-β1), which is associated with

endothelial cells remodelling (Liu et al. 2012). Administration of GSPE also reduced plasmatic ET-1 levels and restored endothelial dysfunction and oxidative stress in both cardiac and renal tissue in L-NAME-induced HTN pregnant mice (Zhu et al. 2018). Moreover, it has been observed an enhancement of eNOS activity and soluble vascular cell adhesion molecule-1 (sVCAM) secretion and a reduction of ET-1 levels and soluble intracellular cell adhesion molecule (sICAM) secretion in HUVEC cells treated with a grape seed extract (Schön et al. 2021a). In addition, GSPE administration has been demonstrated to reduce liver malondialdehyde (MDA), a marker of lipid peroxidation (Quiñones et al. 2013; Pons et al. 2014) and to increase liver GSH levels (Mas-Capdevila et al. 2020) in these animals, which may protect from the oxidative stress associated with HTN. Grape seed extract (200 mg/kg/day between gestational day 10.5 and 18.5) also reduced maternal SBP, plasma MDA levels and improved endothelial-dependent relaxation in mesenteric and uterine arteries of pregnant $eNOS^{-/-}$ mice, an established model of maternal HTN associated with vascular dysfunction (Tropea et al. 2020).

The antihypertensive properties of GSPE have also been demonstrated in hypertensive or pre-hypertensive patients. The results of clinical trials shows that consumption of GSPE (400 mg/kg/day) for 12 weeks could ameliorate vascular stiffness and regulate the BP at a normal stage (Odai et al. 2019). In hypertensive subjects with metabolic syndrome, reduction of oxidative stress by GSPE is one of the main mechanisms involved in BP reduction (Sivaprakasapillai et al. 2009). Volunteers with mild HTN showed a reduction in both SBP and DBP after the intake of 300 mg/day of a grape seed extract but this effect was only observed in men (Schön et al. 2021b). In addition, different meta-analysis studies have demonstrated the positive correlation among grape seed extract consumption and BP reduction. Thus, a meta-analysis with 16 randomized controlled trials found a beneficial impact of a grape seed extract on BP in obese subjects as well as in people with metabolic syndrome (Zhang et al. 2016). Other meta-analysis conducted with 9 randomized controlled trials showed that grape seed phenolic extracts exert their antihypertensive effect over SBP and not over DBP (Feringa et al. 2011). Moreover, it has been proposed that an acute dose of a grape seed extract (300 mg) may be useful to reduce the increase in the risk of cardiovascular events

(increase of blood pressure or vasoconstriction) produced during dynamic exercise in pre-hypertensive men (Kim et al. 2018).

Grape skin extracts

Grape skin extract has shown antioxidant, vasorelaxant and antihypertensive activities. Thus, administration of 100 mg/kg bw of a grape skin extract for 30 days showed significant antihypertensive, vasodilator and antioxidant effects in desoxycortisone acetate (DOCA)-salt and L-NAME-induced hypertensive rats, probably mediated by restoration of NO levels (De Moura et al. 2002). Moreover, administration at 200 mg/kg/day for 12 weeks prevented the development of HTN in SHR due to its antioxidant effect by increasing SOD activity and decreasing MDA levels (De Costa et al. 2020). In other studies, chronic administration of grape skin extract also prevented the development of HTN by increasing NO synthesis and restoring both the activity of SOD, catalase and GPx, as well as MDA levels, in rats with metabolic syndrome induced by a high-fat diet (Emiliano et al. 2011; Resende et al. 2013). Additional studies demonstrated that pre-treatment with grape skin extract prevented the development of HTN in doxorubicin treated rats. This drug induce cardiomyopathy by the overproduction of ROS (Mokni et al. 2012). Furthermore, studies carried out in L-NAME-induced gestional hypertension in rats showed the involvement of NO in the antihypertensive effect of grape skin extract (De Moura et al. 2007). In addition, studies carried out in phenylephrine pre-contracted mesenteric resistance artery rings revealed an endothelial NO-dependent vasorelaxant effect and free radical scavenger activity of two extracts obtained from fermented and non-fermented grape skin (Albuquerque et al. 2017).

Additional studies have revealed that some purinergic components of the sympathetic system can be involved in the effectiveness of grape skin extracts. In this regard, a reduction of SBP was observed in both streptozotocin-induced diabetic and SHR rats (11.2 and 11.8 % respectively) administered a grape skin extract in a dose of 200 mg/day for 3 weeks. A vasorelaxant effect (23% in the maximum of contraction) produced by an increase of the functionality of P1 purinergic receptors (A_1, A_{2A} and A_{2B} subtypes), a reduction of the vasoconstrictor response via decreasing P2 purinergic (P2X1) receptor functionality, and

307 an increase of 13.7 % atrial negative ionotropic effects enhancing the functionality of the P1 purinergic
308 receptor (A_1 subtype) were the mechanisms underlying the bioactivity of the grape skin extract (Bomfim et
309 al. 2019a).

310 ***Grape stem***

311 Stems are also a great source of bioactive compounds which may be used to extract phenolic compounds
312 with both antioxidant and antihypertensive effects (Nieto et al. 2020). In this regard, grape stem extract
313 have showed an IC_{50} of 69.5 µg/mL for *in vitro* ACE inhibitory activity (Lin et al. 2012). In addition,
314 administration of a grape stem extract improved the redox system in endothelial cell cultures increasing
315 GSH and decreasing MDA levels (Goutzourelas et al. 2015). This antioxidant effect was associated to its
316 polyphenols content, being trans-resveratrol, GA, catechin, syringic acid or quercetin the most abundant.
317 Single and chronic dosage of another grape stem extract reduced BP in SHR probably due to their phenolic
318 compounds, including (+)-visitin A. This compound demonstrated *in vitro* ACE inhibitory activity as well
319 as antihypertensive effects in SHR by increasing NO release from endothelial cells (Lin et al. 2012).

320 ***Wine lees***

321 Wine lees (WL) are a great source of phenolic compounds and they have been used to obtain extracts with
322 antioxidants effects (Romero-Díez et al. 2018; Jara-Palacios 2019). It has been evidenced that phenolic
323 compounds identified in WL such as resveratrol, quercetin, GA, (+)-catechin, (−)-epicatechin or malvidin-
324 3-glucoside exhibit antihypertensive effects in hypertensive animal and human models (Quiñones et al.
325 2015; Jin et al. 2017; Marunaka et al. 2017; Fogacci et al. 2019). Considering this evidence, together with
326 the great antioxidant effect of WL extracts and the relationship between oxidative stress and HTN, WL
327 could be a great alternative to manage HTN. Indeed, our group have recently carried out several studies
328 demonstrating the potential of WL and WL-derived products to control HTN for the first time. In this
329 regard, we evaluated the *in vitro* ACE inhibitory activity of the liquid fraction of WL from different grape
330 varieties including Cabernet, Mazuela, and Garnacha grapes, as well as their antihypertensive effects in

SHR at a dose of 5 mL/kg bw. Among these, Cabernet WL exhibited a potent antihypertensive activity, like that obtained with the drug Captopril. This BP-lowering effect was attributed to the presence of flavanols and anthocyanins (López-Fernández-Sobrino et al. 2021c). Three different doses of the Cabernet WL liquid fraction (2.5, 5.0 and 7.5 mL/kg bw) were also studied in SHR after their acute administration. The intermediate dose (5 mL/kg bw) was the most effective to reduce HTN with similar effects than the antihypertensive drug Captopril (50 mg/kg bw) (López-Fernández-Sobrino et al. 2021d). In addition, the antihypertensive effect of the dried WL liquid fraction was also evaluated as alcohol is evaporated during the drying process. A significant enhanced BP-lowering effect of this dried WL fraction was observed in SHR administered a single dose of 125 mg/kg bw (equivalent to 5 mL/kg bw) compared to the corresponding control, being its effect more potent than the one showed by the drug Captopril (López-Fernández-Sobrino et al. 2021d). Moreover, it was discarded a potential hypotensive effect of this WL liquid fraction and its dried product in WKY at the same dose (5 mL/kg bw and 125 mg/kg bw, respectively) (López-Fernández-Sobrino et al. 2021c, 2021d).

In addition, enzyme-assisted extraction was used to obtain a WL product with increased phenolic content and enhanced bioactivities using Flavourzyme® (López-Fernández-Sobrino et al. 2021a). The obtained hydrolysate contained a higher content of phenolic compounds compared to unhydrolyzed WL liquid fraction (1.57 times), being anthocyanins and flavanols the most abundant. This hydrolysate showed greater antioxidant and ACE inhibitory activities and antihypertensive properties than the non-hydrolyzed WL. Moreover, it is worth mentioning that the BP-lowering effect showed by the hydrolyzed WL (5 mL/kg bw) was more prolonged than the one produced by Captopril (50 mg/kg bw) (López-Fernández-Sobrino et al. 2021a). Thus, this study demonstrated that enzymatic hydrolysis is a useful methodology to obtain extracts with enhanced functionalities in WL. The antihypertensive activity of this hydrolysate was also related to its high amount of anthocyanins and flavanols (López-Fernández-Sobrino et al. 2021a). Other additional study showed the potential role of peptides in the antihypertensive effect of this WL hydrolysate. Specifically, the amino acid sequences FKTTDQQTRTTVA, NPKLVTIV, TVTNPARIA,

LDSPSEGRAPG and LDSPSEGRAPGAD were identified in the hydrolysate and showed antihypertensive effect at a single dose of 10 mg/kg bw in SHR (Bravo et al. 2022).

Finally, it was also investigated the mechanisms involved in the antihypertensive effect of the dried Cabernet WL liquid fraction. It was observed an enhanced redox system by the reduction of plasmatic MDA and hepatic ROS and an increase in both plasmatic NO and hepatic GSH levels (López-Fernández-Sobrino et al. 2021d). An additional study demonstrated that the antihypertensive effect of WL powder is mediated by NO and SIRT1. WL phenolic compounds reduced the endothelial expression of *Nox4* and *Et1*, increasing the availability of vascular NO. Furthermore, WL increased *eNos* and *Sirt1* mRNA levels in the endothelium. In addition to those results, the antihypertensive effect of WL was partially mediated by PGI2 (López-Fernández-Sobrino et al. 2021b).

Conclusion

Wine industry by-products may be a valuable option as a source for new alternative natural therapeutics against HTN. In this regard, these products or their derived extracts have shown potent antihypertensive effects, being NO availability one of the main mechanisms involved. Nevertheless, further investigations are needed, especially for some of these by-products such as WL, which have only been tested in animal models. In addition, the use of wine by-products is of particular interest as winery industry generates large amounts of waste that can be reused, as they are rich in phenolic compounds, contributing to a more sustainable environment and to the circular economy.

Author Contributions:

Conceptualization, C.T-F, F.I.B. and B.M.; Funding acquisition, C.T-F, F.I.B and B.M.; Supervision, F.I.B. and B.M.; Writing—Original Draft, R.L-F-S. and C.T-F; Writing—Review & Editing, R.L-F-S., C.T-F, F.I.B and B.M.

Funding:

This work has been supported by the grant number RETOS COLABORACIÓN: RTC-2017-6044-2 from Spanish Ministry of Economy and Competitiveness and European Regional Development Fund (FEDER).

Acknowledgments

F.I.B is a Serra Húnter Fellow.

Conflicts of Interest

The authors declare no conflict of interest.

References

Albuquerque, J.G.F., V.L. Assis, A.J.P.O. Almeida, I.J.L.D. Basílio, M.N. Luciano, B.R.L.A. Meireles, Â.M.T.M. Cordeiro, I.G.A. Araújo, R.C. Veras, T.P. Ribeiro, et al. 2017. Antioxidant and vasorelaxant activities induced by northeastern Brazilian fermented grape skins. *BMC Complementary and Alternative Medicine* 17: 376.

Allam, F., A.T. Dao, G. Chugh, R. Bohat, F. Jafri, G. Patki, C. Mowrey, M. Asghar, K.A. Alkadhi, and S. Salim. 2013. Grape powder supplementation prevents oxidative stress-induced anxiety-like behavior, memory impairment, and high blood pressure in rats. *Journal of Nutrition* 143: 835–842.

Alturfan, A.A., A. Tozan-Beceren, A.Ö. Şehirli, E. Demiralp, G. Şener, and G.Z. Omurtag. 2012. Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. *Molecular Biology Reports* 39: 4589–4596.

Asbaghi, O., F. Naeini, V. Moodi, M. Najafi, M. Shirinbakhshmasoleh, M. Rezaei Kelishadi, A. Hadi, E. Ghaedi, and A. Fadel. 2021a. Effect of grape products on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *International Journal of Food Properties* 24: 627–645.

Asbaghi, O., F. Naeini, V. Moodi, M. Najafi, M. Shirinbakhshmasoleh, M. Rezaei Kelishadi, A. Hadi, E. Ghaedi, and A. Fadel. 2021b. Effect of grape products on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *International Journal of Food Properties* 24: 627–645.

Bagul, P.K., H. Middela, S. Matapally, R. Padiya, T. Bastia, K. Madhusudana, B.R. Reddy, S. Chakravarty, and S.K. Banerjee. 2012. Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. *Pharmacological Research* 66: 260–268.

Barona, J., J.C. Aristizabal, C.N. Blesso, J.S. Volek, and M.L. Fernandez. 2012. Grape polyphenols reduce blood pressure and increase flow-mediated vasodilation in men with metabolic syndrome. *The Journal of Nutrition* 142: 1626–32.

Belcaro, G., A. Ledda, S. Hu, M.R. Cesarone, B. Feragalli, and M. Dugall. 2013. Grape seed procyanidins in pre- and mild hypertension: A registry study. *Evidence-Based Complementary and Alternative Medicine* 2013: 313142.

Bell, D.R., and K. Gochenaur. 2006. Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *Journal of Applied Physiology* 100: 1164–1170.

Bhatt, S.R., M.F. Lokhandwala, and A.A. Banday. 2011. Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. *European Journal of Pharmacology* 667: 258–264.

Bomfim, G.H.S., D.C. Musial, R. Miranda-Ferreira, S.R. Nascimento, A. Jurkiewicz, N.H. Jurkiewicz, and R.S. de Moura. 2019a. Antihypertensive effects of the Vitis vinifera grape skin (ACH09) extract consumption elicited by functional improvement of P1 (A1) and P2 (P2X1) purinergic receptors in diabetic and hypertensive rats. *PharmaNutrition* 8: 100146.

Bomfim, G.H.S., D.C. Musial, R. Miranda-Ferreira, S.R. Nascimento, A. Jurkiewicz, N.H. Jurkiewicz, and R.S. de Moura. 2019b. Antihypertensive effects of the Vitis vinifera grape skin (ACH09) extract consumption elicited by functional improvement of P1 (A1) and P2 (P2X1) purinergic receptors in diabetic and hypertensive rats. *PharmaNutrition* 8: 100146.

Bonnefont-Rousselot, D. 2016. Resveratrol and cardiovascular diseases. *Nutrients* 8: 250.

Borghi, A. Del, L. Moreschi, and M. Gallo. 2020. Circular economy approach to reduce water–energy–food nexus. *Current Opinion in Environmental Science and Health* 13: 23–28.

Bravo, F.I., A. Mas-Capdevila, R. López-Fernández-Sobrino, C. Torres-Fuentes, M. Mulero, J.M. Alcaide-Hidalgo, and B. Muguerza. 2022. Identification of novel antihypertensive peptides from wine lees hydrolysate. *Food Chemistry* 366: 130690.

Brüll, V., C. Burak, B. Stoffel-Wagner, S. Wolffram, G. Nickenig, C. Müller, P. Langguth, B. Alteheld, R. Fimmers, S. Naaf, et al. 2015. Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-)hypertension: A randomised double-blinded placebo-controlled cross-over trial. *British Journal of Nutrition* 114: 1263–1277.

Cachofeiro, V., M. Miana, N. de las Heras, B. Martín-Fernández, S. Ballesteros, J. Fernández-Tresguerres, and V. Lahera. 2008. Aldosterone and the vascular system. *The Journal of Steroid Biochemistry and Molecular Biology* 109: 331–335.

Calfío, C., and J.P. Huidobro-Toro. 2019. Potent vasodilator and cellular antioxidant activity of endemic patagonian calafate berries (berberis microphylla) with nutraceutical potential. *Molecules* 24: 2700.

Cheng, C.K., J. Luo, C.W. Lau, Z. Chen, X.Y. Tian, and Y. Huang. 2020. Pharmacological basis and new insights of resveratrol action in the cardiovascular system. *British Journal of Pharmacology* 177: 1258–1277.

Cheng, Y.C., J.M. Sheen, W.L. Hu, and Y.C. Hung. 2017. Polyphenols and Oxidative Stress in Atherosclerosis-Related Ischemic Heart Disease and Stroke. *Oxidative Medicine and Cellular Longevity* 2017.

Chiva-Blanch, G., M. Urpi-Sarda, E. Ros, S. Arranz, P. Valderas-Martínez, R. Casas, E. Sacanella, R. Llorach, R.M. Lamuela-Raventos, C. Andres-Lacueva, et al. 2012. Dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide: Short communication. *Circulation*

Research 111: 1065–1068.

Cienfuegos-Jovellanos, E., M. Del Mar Quiñones, B. Muguerza, L. Moulay, M. Miguel, and A. Aleixandre. 2009. Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. *Journal of Agricultural and Food Chemistry* 57: 6156–6162.

Collier, S.R., and M.J. Landram. 2012. Treatment of prehypertension: Lifestyle and/or medication. *Vascular Health and Risk Management* 8: 613–619.

Costa, G.F. De, D.T. Ognibene, C.A. Da Costa, M.T. Teixeira, V. Da Silva Cristino Cordeiro, G.F. De Bem, A.S. Moura, A. De Castro Resende, and R.S. De Moura. 2020. Vitis vinifera L grape skin extract prevents development of hypretension and altered lipid profile in sponteneously hypertensive tats: Role of oxidative stress. *Preventive Nutrition and Food Science* 25: 25–31.

Crowley, S.D. 2014. The Cooperative roles of inflammation and oxidative stress in the pathogenesis of hypertension. *Antioxidants and Redox Signaling* 20: 102–120.

Dalton, T.P., Y. Chen, S.N. Schneider, D.W. Nebert, and H.G. Shertzer. 2004. Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radical Biology and Medicine* 37: 1511–1526.

Dohadwala, M.M., and J.A. Vita. 2009. Grapes and cardiovascular disease. *The Journal of Nutrition* 139: 1788S-1793S.

Egert, S., A. Bosy-Westphal, J. Seiberl, C. Kürbitz, U. Settler, S. Plachta-Danielzik, A.E. Wagner, J. Frank, J. Schrezenmeir, G. Rimbach, et al. 2009. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. *British Journal of Nutrition* 102: 1065–1074.

Emiliano, A.F., L.C.R.M. de Cavalho, V. da Silva Cristino Cordeiro, C.A. da Costa, P.B.R. de Oliveira, E.F. Queiroz, D.D. Col Moreira, G.T. Boaventura, R.S. de Moura, and A.C. Resende. 2011. Metabolic

471 disorders and oxidative stress programming in offspring of rats fed a high-fat diet during lactation: Effects
472 of a vinifera grape skin (ACH09) extract. *Journal of Cardiovascular Pharmacology* 58: 319–328.

473 Fairlie-Jones, L., K. Davison, E. Fromentin, and A. Hill. 2017. The effect of anthocyanin-rich foods or
474 extracts on vascular function in adults: A systematic review and meta-analysis of randomised controlled
475 trials. *Nutrients* 9: 908.

476 Feringa, H.H.H., D.A. Laskey, J.E. Dickson, and C.I. Coleman. 2011. The effect of grape seed extract on
477 cardiovascular risk markers: A meta-analysis of randomized controlled trials. *Journal of the American*
478 *Dietetic Association* 111: 1173–1181.

479 Fogacci, F., G. Tocci, V. Presta, A. Fratter, C. Borghi, and A.F.G. Cicero. 2019. Effect of resveratrol on
480 blood pressure: A systematic review and meta-analysis of randomized, controlled, clinical trials. *Critical*
481 *Reviews in Food Science and Nutrition* 59: 1605–1618.

482 Food and Agriculture Organization of the United Nations. 2020. *Food wastage: Key facts and figures*.
483 https://www.fao.org/news/story/en/item/196402/icode/.

484 Forman, H.J., H. Zhang, and A. Rinna. 2009. Glutathione: Overview of its protective roles, measurement,
485 and biosynthesis. *Molecular Aspects of Medicine* 30: 1–12.

486 Franco, J.G., P.C. Lisboa, N.S. Lima, T.A.S. Amaral, N. Peixoto-Silva, A.C. Resende, E. Oliveira, M.C.F.
487 Passos, and E.G. Moura. 2013. Resveratrol attenuates oxidative stress and prevents steatosis and
488 hypertension in obese rats programmed by early weaning. *Journal of Nutritional Biochemistry* 24: 960–
489 966.

490 Gerardi, G., M. Cavia-Saiz, R. del Pino-García, M.D. Rivero-Pérez, M.L. González-SanJosé, and P. Muñiz.
491 2020a. Wine pomace product ameliorates hypertensive and diabetic aorta vascular remodeling through
492 antioxidant and anti-inflammatory actions. *Journal of Functional Foods* 66: 103794.

493 Gerardi, G., M. Cavia-Saiz, R. del Pino-García, M.D. Rivero-Pérez, M.L. González-SanJosé, and P. Muñiz.

494 2020b. Wine pomace product ameliorates hypertensive and diabetic aorta vascular remodeling through
495 antioxidant and anti-inflammatory actions. *Journal of Functional Foods* 66: 103794.

496 Gertz, M., G.T.T. Nguyen, F. Fischer, B. Suenkel, C. Schlicker, B. Fränzel, J. Tomaschewski, F. Aladini,
497 C. Becker, D. Wolters, et al. 2012. A molecular mechanism for direct sirtuin activation by resveratrol. *PLoS*
498 *ONE* 7: e49761.

499 Goutzourelas, N., D. Stagos, A. Housmekeridou, C. Karapouliou, E. Kerasioti, N. Aligiannis, A.L.
500 Skaltsounis, D.A. Spandidos, A.M. Tsatsakis, and D. Kouretas. 2015. Grape pomace extract exerts
501 antioxidant effects through an increase in GCS levels and GST activity in muscle and endothelial cells.
502 *International Journal of Molecular Medicine* 36: 433–441.

503 Guerrero, L., J. Castillo, M. Quiñones, S. Garcia-Vallvé, L. Arola, G. Pujadas, and B. Muguerza. 2012.
504 Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies.
505 *PLoS ONE* 7: e49493.

506 Gupta, M., S. Dey, D. Marbaniang, P. Pal, S. Ray, and B. Mazumder. 2020. Grape seed extract: having a
507 potential health benefits. *Journal of Food Science and Technology* 57: 1205–1215.

508 Huang, H., G. Chen, D. Liao, Y. Zhu, and X. Xue. 2016. Effects of berries consumption on cardiovascular
509 risk factors: A meta-analysis with trial sequential analysis of randomized controlled trials. *Scientific Reports*
510 6: 23625.

511 Iglesias-Carres, L., A. Mas-Capdevila, L. Sancho-Pardo, F.I. Bravo, M. Mulero, B. Muguerza, and A.
512 Arola-Arnal. 2018. Optimized extraction by response surface methodology used for the characterization
513 and quantification of phenolic compounds in whole red grapes (Vitis vinifera). *Nutrients* 10: 1931.

514 International Organisation of Vine and Wine. 2019. 2019 Statistical report on world vitiviniculture.

515 Jara-Palacios, M.J. 2019. Wine Lees as a source of antioxidant compounds. *Antioxidants* 8: 45.

516 Jiménez, J.P., J. Serrano, M. Tabernero, S. Arranz, M.E. Díaz-Rubio, L. García-Diz, I. Goñi, and F. Saura-

517 Calixto. 2008. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. *Nutrition* 24:
518 646–53.

519 Jin, L., Z.H. Piao, S. Sun, B. Liu, G.R. Kim, Y.M. Seok, M.Q. Lin, Y. Ryu, S.Y. Choi, H.J. Kee, et al. 2017.
520 Gallic acid reduces blood pressure and attenuates oxidative stress and cardiac hypertrophy in spontaneously
521 hypertensive rats. *Scientific Reports* 7: 15607.

522 Kang, N., J.H. Lee, W.W. Lee, J.Y. Ko, E.A. Kim, J.S. Kim, M.S. Heu, G.H. Kim, and Y.J. Jeon. 2015.
523 Gallic acid isolated from Spirogyra sp. improves cardiovascular disease through a vasorelaxant and
524 antihypertensive effect. *Environmental Toxicology and Pharmacology* 39: 764–772.

525 Kappagoda. 2012. Effect of Grape Seed Extract on Blood Pressure in Subjects with Pre-Hypertension.
526 *Journal of Pharmacy and Nutrition Sciences*.

527 Kim, J.-K., K.-A. Kim, H.-M. Choi, S.-K. Park, and C.L. Stebbins. 2018. Grape Seed Extract
528 Supplementation Attenuates the Blood Pressure Response to Exercise in Prehypertensive Men. *Journal of*
529 *Medicinal Food* 21: 445–453.

530 Kim, J.N., S.N. Han, T.J. Ha, and H.K. Kim. 2017. Black soybean anthocyanins attenuate inflammatory
531 responses by suppressing reactive oxygen species production and mitogen activated protein kinases
532 signaling in lipopolysaccharide-stimulated macrophages. *Nutrition Research and Practice* 11: 357–364.

533 Kiviniemi, T.O., A. Saraste, T. Lehtimäki, J.O. Toikka, M. Saraste, O.T. Raitakari, J.J. Hartiala, J. Viikari,
534 and J.W. Koskenvuo. 2010. Decreased endothelin-1 levels after acute consumption of red wine and de-
535 alcoholized red wine. *Atherosclerosis* 211: 283–286.

536 Konior, A., A. Schramm, M. Czesnikiewicz-Guzik, and T.J. Guzik. 2014. NADPH oxidases in vascular
537 pathology. *Antioxidants & Redox Signaling* 20: 2794–2814.

538 Larson, A.J., J. David Symons, and T. Jalili. 2012. Therapeutic potential of quercetin to decrease blood
539 pressure: Review of efficacy and mechanisms. *Advances in Nutrition* 3: 39–46.

Leikert, J.F., T.R. Räthel, P. Wohlfart, V. Cheynier, A.M. Vollmar, and V.M. Dirsch. 2002. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation* 106: 1614–1617.

Li, S.H., P. Zhao, H.B. Tian, L.H. Chen, and L.Q. Cui. 2015. Effect of grape polyphenols on blood pressure: A meta-analysis of randomized controlled trials. *PLoS ONE* 10: e0137665.

Lin, Y.S., Y.L. Lu, G.J. Wang, L.G. Chen, C.L. Wen, and W.C. Hou. 2012. Ethanolic extracts and isolated compounds from small-leaf grape (Vitis thunbergii var. taiwaniana) with antihypertensive activities. *Journal of Agricultural and Food Chemistry* 60: 7435–7441.

Liu, X., J. Qiu, S. Zhao, B. You, X. Ji, Y. Wang, X. Cui, Q. Wang, and H. Gao. 2012. Grape seed proanthocyanidin extract alleviates ouabain-induced vascular remodeling through regulation of endothelial function. *Molecular Medicine Reports* 6: 949–954.

Loke, W.M., J.M. Hodgson, J.M. Proudfoot, A.J. McKinley, I.B. Puddey, and K.D. Croft. 2008. Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *American Journal of Clinical Nutrition* 88: 1018–1025.

López-Fernández-Sobrino, R., M. Margalef, C. Torres-Fuentes, J. Ávila-Román, G. Aragonès, B. Muguerza, and F.I. Bravo. 2021a. Enzyme-assisted extraction to obtain phenolic-enriched wine lees with enhanced bioactivity in hypertensive rats. *Antioxidants* 10: 517.

López-Fernández-Sobrino, R., J.R. Soliz-Rueda, J. Ávila-Román, A. Arola-Arnal, M. Suárez, B. Muguerza, and F.I. Bravo. 2021b. Blood pressure-lowering effect of wine lees phenolic compounds is mediated by endothelial-derived factors: Role of sirtuin 1. *Antioxidants* 10: 1073.

López-Fernández-Sobrino, R., J.R. Soliz-Rueda, M. Margalef, A. Arola-Arnal, M. Suárez, F.I. Bravo, and B. Muguerza. 2021c. ACE inhibitory and antihypertensive activities of wine lees and relationship among bioactivity and phenolic profile. *Nutrients* 13: 679.

López-Fernández-Sobrino, R., J.R. Soliz-Rueda, M. Suárez, M. Mulero, L. Arola, F.I. Bravo, and B. Muguerza. 2021d. Blood pressure-lowering effect of wine lees: Dose-response study, effect of dealcoholization and possible mechanisms of action. *Nutrients* 13: 1142.

Luo, M., R. Tian, Z. Yang, Y.Y. Peng, and N. Lu. 2019. Quercetin suppressed NADPH oxidase-derived oxidative stress via heme oxygenase-1 induction in macrophages. *Archives of Biochemistry and Biophysics* 671: 69–76.

Mancia, G., and A. Zanchetti. 2008. Choice of antihypertensive drugs in the European Society of Hypertension–European Society of Cardiology guidelines: specific indications rather than ranking for general usage. *Journal of Hypertension* 26: 164–168.

Margalef, M., F.I. Bravo, A. Arola-Arnal, and B. Muguerza. 2017. Natural angiotensin converting enzyme (ACE) inhibitors with antihypetensive properties In: Andrade, P.., P. Valentao, and D.M. Pereira, Eds., Natural Products Targeting Clinically Relevant Enzymes. Weinheim: Wiley-VCH Verlag GmbH & Co., pp. 45–67.

Marunaka, Y., R. Marunaka, H. Sun, T. Yamamoto, N. Kanamura, T. Inui, and A. Taruno. 2017. Actions of quercetin, a polyphenol, on blood pressure. *Molecules* 22: 209.

Mas-Capdevila, A., L. Iglesias-Carres, A. Arola-Arnal, M. Suárez, F.I. Bravo, and B. Muguerza. 2020. Changes in arterial blood pressure caused by long-term administration of grape seed proanthocyanidins in rats with established hypertension. *Food & Function* 11: 8735–8742.

Masaki, T., and T. Sawamura. 2006. Endothelin and endothelial dysfunction. *Proceedings of the Japan Academy, Series B* 82: 17–24.

Mellen, P.B., K.R. Daniel, K.B. Brosnihan, K.J. Hansen, and D.M. Herrington. 2010. Effect of muscadine grape seed supplementation on vascular function in subjects with or at risk for cardiovascular disease: a randomized crossover trial. *Journal of the American College of Nutrition* 29: 469–75.

Miatello, R., M. Vázquez, N. Renna, M. Cruzado, A.P. Zumino, and N. Risler. 2005. Chronic administration of resveratrol prevents biochemical cardiovascular changes in fructose-fed rats. *American Journal of Hypertension* 18: 864–870.

Mizutani, K., K. Ikeda, Y. Kawai, and Y. Yamori. 2000. Resveratrol attenuates ovariectomy-induced hypertension and bone loss in stroke-prone spontaneously hypertensive rats. *Journal of Nutritional Science and Vitaminology* 46: 78–83.

Mokni, M., S. Hamlaoui-Guesmi, M. Amri, L. Marzouki, F. Limam, and E. Aouani. 2012. Grape seed and skin extract protects against acute chemotherapy toxicity induced by doxorubicin in rat heart. *Cardiovascular Toxicology* 12: 158–165.

Moura, R.S. De, Â.C. Resende, A.S. Moura, and M.F. Maradei. 2007. Protective action of a hydroalcoholic extract of a vinifera grape skin on experimental preeclampsia in rats. *Hypertension in Pregnancy* 26: 89–100.

Moura, R.S. De, F.S.C. Viana, M.A. V. Souza, K. Kovary, D.C. Guedes, E.P.B. Oliveira, L.M.S. Rubenich, L.C.R.M. Carvalho, R.M. Oliveira, T. Tano, et al. 2002. Antihypertensive, vasodilator and antioxidant effects of a vinifera grape skin extract. *Journal of Pharmacy and Pharmacology* 54: 1515–1520.

Muntner, P., and P.K. Whelton. 2017. Using predicted cardiovascular disease risk in conjunction with blood pressure to guide antihypertensive medication treatment. *Journal of the American College of Cardiology* 69: 2446–2456.

Nieto, J.A., S. Santoyo, M. Prodanov, G. Reglero, and L. Jaime. 2020. Valorisation of grape stems as a source of phenolic antioxidants by using a sustainable extraction methodology. *Foods* 9: 604.

Odai, T., M. Terauchi, K. Kato, A. Hirose, and N. Miyasaka. 2019. Effects of grape seed proanthocyanidin extract on vascular endothelial function in participants with prehypertension: A randomized, double-blind, placebo-controlled study. *Nutrients* 11: 2844.

609 Park, E., I. Edirisinghe, Y.Y. Choy, A. Waterhouse, and B. Burton-Freeman. 2016. Effects of grape seed
610 extract beverage on blood pressure and metabolic indices in individuals with pre-hypertension: A
611 randomised, double-blinded, two-arm, parallel, placebo-controlled trial. *British Journal of Nutrition* 115:
612 226–238.

613 Pechánová, O., I. Bernátová, P. Babál, M.C. Martínez, S. Kyselá, S. Stvrtina, and R. Andriantsitohaina.
614 2004. Red wine polyphenols prevent cardiovascular alterations in L-NAME-induced hypertension. *Journal*
615 *of Hypertension* 22: 1551–1559.

616 Pino-García, R. Del, M.D. Rivero-Pérez, M.L. González-Sanjosé, K.D. Croft, and P. Muñiz. 2017.
617 Antihypertensive and antioxidant effects of supplementation with red wine pomace in spontaneously
618 hypertensive rats. *Food and Function* 8: 2444–2454.

619 Pons, Z., L. Guerrero, M. Margalef, L. Arola, A. Arola-Arnal, and B. Muguerza. 2014. Effect of low
620 molecular grape seed proanthocyanidins on blood pressure and lipid homeostasis in cafeteria diet-fed rats.
621 *Journal of Physiology and Biochemistry* 70: 629–637.

622 Pons, Z., M. Margalef, F.I. Bravo, A. Arola-Arnal, and B. Muguerza. 2016a. Acute administration of single
623 oral dose of grape seed polyphenols restores blood pressure in a rat model of metabolic syndrome: role of
624 nitric oxide and prostacyclin. *European Journal of Nutrition* 55: 749–758.

625 Pons, Z., M. Margalef, F.I. Bravo, A. Arola-Arnal, and B. Muguerza. 2016b. Grape seed flavanols decrease
626 blood pressure via Sirt-1 and confer a vasoprotective pattern in rats. *Journal of Functional Foods* 24: 164–
627 172.

628 Pons, Z., M. Margalef, F.I. Bravo, A. Arola-Arnal, and B. Muguerza. 2017. Chronic administration of
629 grape-seed polyphenols attenuates the development of hypertension and improves other cardiometabolic
630 risk factors associated with the metabolic syndrome in cafeteria diet-fed rats. *British Journal of Nutrition*
631 117: 200–208.

Queipo-Ortuño, M.I., M. Boto-Ordóñez, M. Murri, J.M. Gomez-Zumaquero, M. Clemente-Postigo, R. Estruch, F. Cardona Diaz, C. Andrés-Lacueva, and F.J. Tinahones. 2012. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *The American Journal of Clinical Nutrition* 95: 1323–1334.

Quiñones, M., L. Guerrero, S. Fernández-Vallinas, Z. Pons, L. Arola, A. Aleixandre, and B. Muguerza. 2014. Involvement of nitric oxide and prostacyclin in the antihypertensive effect of low-molecular-weight procyanidin rich grape seed extract in male spontaneously hypertensive rats. *Journal of Functional Foods* 6: 419–427.

Quiñones, M., L. Guerrero, M. Suarez, Z. Pons, A. Aleixandre, L. Arola, and B. Muguerza. 2013. Low-molecular procyanidin rich grape seed extract exerts antihypertensive effect in males spontaneously hypertensive rats. *Food Research International* 51: 587–595.

Quiñones, M., M. Margalef, A. Arola-Arnal, B. Muguerza, M. Miguel, and A. Aleixandre. 2015. The blood pressure effect and related plasma levels of flavan-3-ols in spontaneously hypertensive rats. *Food and Function* 6: 3479–3489.

Quiñones, M., M. Miguel, B. Muguerza, and A. Aleixandre. 2011. Effect of a cocoa polyphenol extract in spontaneously hypertensive rats. *Food and Function* 2: 649–653.

Ramar, M., B. Manikandan, T. Raman, A. Priyadarsini, S. Palanisamy, M. Velayudam, A. Munusamy, N. Marimuthu Prabhu, and B. Vaseeharan. 2012. Protective effect of ferulic acid and resveratrol against alloxan-induced diabetes in mice. *European Journal of Pharmacology* 690: 226–235.

Rasines-Perea, Z., I. Ky, G. Cros, A. Crozier, and P.-L. Teissedre. 2018. Grape pomace: Antioxidant activity, potential effect against hypertension and metabolites characterization after intake. *Diseases* 6: 60.

Resende, A.C., A.F. Emiliano, V.S.C. Cordeiro, G.F. de Bem, L.C.R.M. de Cavalho, P.R.B. de Oliveira, M.L. Neto, C.A. Costa, G.T. Boaventura, and R.S. de Moura. 2013. Grape skin extract protects against

programmed changes in the adult rat offspring caused by maternal high-fat diet during lactation. *Journal of Nutritional Biochemistry* 24: 2119–2126.

Rodrigo, R., R. Brito, G. Castillo, J. González, and N. Valls. 2015. Oxidative stress in hypertension: Mechanisms and therapeutic opportunities. *Experimental and Clinical Endocrinology Diabetes* 123: 325–335.

Rodriguez-Mateos, A., G. Istas, L. Boschek, R.P. Feliciano, C.E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic, and C. Heiss. 2019. Circulating anthocyanin metabolites mediate vascular benefits of blueberries: Insights from randomized controlled trials, metabolomics, and nutrigenomics. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 74: 967–976.

Romero-Díez, R., S. Rodríguez-Rojo, M.J. Cocero, C.M.M. Duarte, A.A. Matias, and M.R. Bronze. 2018. Phenolic characterization of aging wine lees: Correlation with antioxidant activities. *Food Chemistry* 259: 188–195.

Sano, A., R. Uchida, M. Saito, N. Shioya, Y. Komori, Y. Tho, and N. Hashizume. 2007. Beneficial effects of grape seed extract on malondialdehyde-modified LDL. *Journal of Nutritional Science and Vitaminology* 53: 174–182.

Sarr, M., M. Chataigneau, S. Martins, C. Schott, J. El Bedoui, M.H. Oak, B. Muller, T. Chataigneau, and V.B. Schini-Kerth. 2006. Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: Role of NADPH oxidase. *Cardiovascular Research* 71: 794–802.

Schön, C., P. Allegrini, K. Engelhart-Jentzsch, A. Riva, and G. Petrangolini. 2021a. Grape Seed Extract Positively Modulates Blood Pressure and Perceived Stress: A Randomized, Double-Blind, Placebo-Controlled Study in Healthy Volunteers. *Nutrients* 13: 654.

Schön, C., P. Allegrini, K. Engelhart-Jentzsch, A. Riva, and G. Petrangolini. 2021b. Grape Seed Extract Positively Modulates Blood Pressure and Perceived Stress: A Randomized, Double-Blind, Placebo-

678 Controlled Study in Healthy Volunteers. *Nutrients* 13: 654.

679 Serban, M.C., A. Sahebkar, A. Zanchetti, D.P. Mikhailidis, G. Howard, D. Antal, F. Andrica, A. Ahmed,
680 W.S. Aronow, P. Muntner, et al. 2016. Effects of quercetin on blood pressure: A systematic review and
681 meta-analysis of randomized controlled trials. *Journal of the American Heart Association* 5: e002713.

682 Shrikhande, A.J. 2000. Wine by-products with health benefits. *Food Research International* 33: 469–474.

683 Sivaprakasapillai, B., I. Edirisinghe, J. Randolph, F. Steinberg, and T. Kappagoda. 2009. Effect of grape
684 seed extract on blood pressure in subjects with the metabolic syndrome. *Metabolism: Clinical and*
685 *Experimental* 58: 1743–1746.

686 Snopek, L., J. Mlcek, L. Sochorova, M. Baron, I. Hlavacova, T. Jurikova, R. Kizek, E. Sedlackova, and J.
687 Sochor. 2018. Contribution of red wine consumption to human health protection. *Molecules* 23: 1–16.

688 Sturrock, E.D., L. Lubbe, G.E. Cozier, S.L.U. Schwager, A.T. Arowolo, L.B. Arendse, E. Belcher, and
689 K.R. Acharya. 2019. Structural basis for the C-domain-selective angiotensin-converting enzyme inhibition
690 by bradykinin-potentiating peptide b (BPPb). *Biochemical Journal* 476: 1553–1570.

691 Taladrid, D., M. de Celis, I. Belda, B. Bartolome, and M.V. Moreno-Arribas. 2022a. Hypertension- and
692 glycaemia-lowering effects of a grape-pomace-derived seasoning in patients and healthy volunteers.
693 Interplay with the gut microbiome. *Food & Function*.

694 Taladrid, D., M. de Celis, I. Belda, B. Bartolome, and M.V. Moreno-Arribas. 2022b. Hypertension- and
695 glycaemia-lowering effects of a grape-pomace-derived seasoning in patients and healthy volunteers.
696 Interplay with the gut microbiome. *Food & Function*.

697 Terauchi, M., N. Horiguchi, A. Kajiyama, M. Akiyoshi, Y. Owa, K. Kato, and T. Kubota. 2014. Effects of
698 grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular
699 parameters in middle-aged women. *Menopause* 21: 990–996.

700 Thandapilly, S.J., J.L. Lemaistre, X.L. Louis, C.M. Anderson, T. Netticadan, and H.D. Anderson. 2012.

Vascular and cardiac effects of grape powder in the spontaneously hypertensive rat. *American Journal of Hypertension* 25: 1070–1076.

Thomopoulos, C., G. Parati, and A. Zanchetti. 2014. Effects of blood pressure lowering on outcome incidence in hypertension.1. Overview, meta-analyses, and meta-regression analyses of randomized trials. *Journal of Hypertension* 32: 2285–2295.

Tropea, T., S.L. Greenwood, C.P. Sibley, and E.C. Cottrell. 2020. Grape seed extract polyphenols improve resistance artery function in pregnant eNOS–/– mice. *Frontiers in Physiology* 11: 588000.

Unger, T., C. Borghi, F. Charchar, N.A. Khan, N.R. Poulter, D. Prabhakaran, A. Ramirez, M. Schlaich, G.S. Stergiou, M. Tomaszewski, et al. 2020. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. *Hypertension* 75: 1334–1357.

Urquiaga, I., S. D'Acuña, D. Pérez, S. Dicenta, G. Echeverría, A. Rigotti, and F. Leighton. 2015. Wine grape pomace flour improves blood pressure, fasting glucose and protein damage in humans: A randomized controlled trial. *Biological Research* 48: 49.

Ward, N.C., J.M. Hodgson, K.D. Croft, V. Burke, L.J. Beilin, and I.B. Puddey. 2005. The combination of vitamin C and grape-seed polyphenols increases blood pressure: a randomized, double-blind, placebo-controlled trial. *Journal of Hypertension* 23: 427–34.

World Health Organization. 2021. *Hypertension*. https://www.who.int/news-room/fact-sheets/detail/hypertension.

Xia, N., A. Daiber, U. Förstermann, and H. Li. 2017. Antioxidant effects of resveratrol in the cardiovascular system. *British Journal of Pharmacology* 174: 1633–1646.

Zhang, H., S. Liu, L. Li, S. Liu, S. Liu, J. Mi, and G. Tian. 2016. The impact of grape seed extract treatment on blood pressure changes: A meta-analysis of 16 randomized controlled trials. *Medicine* 95: e4247.

Zhu, F., J. Hu, F. Lv, P. Cheng, and S. Gao. 2018. Effects of oligomeric grape seed proanthocyanidins on

724 *L*- NAME - induced hypertension in pregnant mice: Role of oxidative stress and endothelial dysfunction.

725 *Phytotherapy Research* 32: 1836–1847.

726

727

728 Table 1. Antihypertensive effects of grapes, wine and winemaking by-products in different animal models and molecular mechanisms
729 involved in their bioactivity.

Grape source	Extraction condition/ Manufacturer	Experimental model	Dose (mg/kg bw)	Administration duration	ΔSBP/DBP	Action mechanism*	Ref.
Whole grape powder	none	Sprague-Dawley rats with BSO	300	3 weeks	20/20 %	↓ ERK-1/2	(Allam et al. 2013)
Whole grape powder	none	SHR	600	10 weeks	8 /11 mmHg	n.d.	(Thandapilly et al. 2012)
Red wine	none	Wistar rats with Ang II	150	20 days	~20 mmHg/n.d.	↑ Renin ↓ NOX ↓ ROS	(Sarr et al. 2006)
Red wine polyphenols	Mr D. Ageron (Société Francaise de Distillerie, Vallont Pont d'Arc, France)	Wistar rats with L-NAME	40	4 weeks	18%/n.d.	↑ eNOS, NOS activity	(Pechánová et al. 2004)
Red pomace	Patent method	SHR	300	4 weeks	n.d	↑ aorta phosphorylated eNOS ↓ aorta ROS	(Gerardi et al. 2020b)
Red pomace	High temperature (50 ºC) and alcoholic extraction (70:30, Ethanol:Water, v/v)	SHR	25	4 weeks	~25 mmHg/n.d.	n.d.	(Rasines-Perea et al. 2018)

Red pomace	Patent method	SHR	300	4 weeks	30 mmHg/n.d.	↑ NO, *Ho-1*, *Sod2* ↓ *Ace,* MDA	(Del Pino-García et al. 2017)
Skin	High temperature (100ºC) and alcoholic extraction (1:1, Ethanol:Water, v/v)	SHR	200	12 weeks	~60 mmHg/n.d.	↑ SOD ↓ TBARS	(De Costa et al. 2020)
Skin	Aqueous solution (100ºC, 120 min)+ ethanol and ethanol/H_2O (1:1) fractions from ion-exchange resin column	SHR	200	21 days	11.8 %/-	↓RA and LA ↑NIE via P1 (A_1 subtype) receptor ↓PIE via P2 receptor	(Bomfim et al. 2019b)
Skin	Aqueous solution (100ºC, 120 min)+ ethanol and ethanol/H_2O (1:1) fractions from ion-exchange resin column	Streptozotocin-induced diabetic rats	200	21 days	11.2 %/-	↑NIE via P1 (A_1 subtype) receptor ↓PIE via P2 receptor ↓P2 (P2X1) receptor functionality	
Skin	High temperature (100ºC) and alcoholic extraction (1:1,	Wistar rats with high fat diet	200	180 days	~30 mmHg/n.d.	↑ NO ↑ SOD, CAT, GPx activities ↓ MDA	(Emiliano et al. 2011; Resende

	Ethanol:Water, v/v)						et al. 2013)
Skin	Alcoholic extraction (1:1, Ethanol:Water, v/v)	Pregnancy female rats	200	Chronic	n.d./n.d.	NO involvement	(De Moura et al. 2007)
Skin	Alcoholic extraction (50:50, Ethanol:Water, v/v)	Wistar rats with L-NAME + GSE	100	4 weeks	28/12 mmHg	Implication of NO ↓ MDA	(De Moura et al. 2002)
Skin	Alcoholic extraction (50:50, Ethanol:Water, v/v)	DOCA-salt + GSE	100	4 weeks	25/57 mmHg	Implication of NO ↓ MDA	
Seed	MegaNatural® BP; Polyphenolics	Pregnant eNOS$^{-/-}$	200	Gestacional day 10.5-18.5	↓SBP	↓ MDA Endothelium-dependent relaxation	(Tropea et al. 2020)
Seed	Les Dérives Résiniques et Terpéniques	CHR	25	3 weeks	18/17 mmHg	↑ GSH, *Sirt1* ↓*Et1*	(Mas-Capdevila et al. 2020)
Seed	JF-natural Corporation (Tianjin, China)	Kunming mice with L-NAME	-	3 weeks	~20/ ~10 mmHg	↑ eNOS, NO, SOD ↓ MDA, ET-1	(Zhu et al. 2018)
Seed	Les Dérives Résiniques et Terpéniques	CHR	25	12 weeks	-/-	n.d.	(Pons et al. 2017)
Seed	Les Dérives Résiniques et Terpéniques	CHR	100	12 weeks	-/-	n.d.	
Seed	Les Dérives Résiniques et Terpéniques	CHR	200	12	-/-	n.d.	

Seed	Les Dérives Résiniques et Terpéniques	CHR	375	Acute	21/22 mmHg	Involvement of NO and PGI2	(Pons et al. 2016a)
Seed	Les Dérives Résiniques et Terpéniques	CHR	375	Acute	21/22 mmHg	↑ e*Nos, Sirt1* ↓ *Nox4, Et1*	(Pons et al. 2016b)
Seed	Les Dérives Résiniques et Terpéniques	SHR	375	Acute	-/-	Involvement of SIRT1	
Seed	Les Dérives Résiniques et Terpéniques	SHR	375	Acute	35.7/34.5 mmHg	↑ PGFα1, Involvement of NO and PGI2	(Quiñones et al. 2014)
Seed	Les Dérives Résiniques et Terpéniques	CHR	375	Acute	~18/~13 mmHg	↓ MDA	(Pons et al. 2014)
Seed	Les Dérives Résiniques et Terpéniques (Dax, France)	SHR	375	Acute	48/57.4 mmHg	↑ GSH	(Quiñones et al. 2013)
Seed	Proanthocyanid in extract	Sprague-Dawley rats with ouabain	250	5 weeks	38.5 mmHg/-	↑ NO ↓ ET-1, *Tgf-β1*	(Liu et al. 2012)
Cabernet wine lees	Liquid fraction	SHR	5 mL/kg bw	Acute	32.5/32.2 mmHg	n.d.	(López-Fernández-Sobrino et al. 2021a)
Cabernet wine lees	Hydrolysis with Flavourzyme	SHR	5 mL/kg bw	Acute	35.6/35.2 mmHg	n.d.	
Cabernet wine lees	Liquid fraction	SHR	5 mL/kg bw	Acute	36.4/38.8 mmHg	n.d.	(López-Fernández-Sobrino et al. 2021c)
Cabernet wine lees	Dried liquid fraction	SHR	125	Acute	28/30 mmHg	↑*Sirt1, eNos* ↓ *Et1, Nox4*	(López-Fernández

Cabernet wine lees	Dried liquid fraction	SHR+L-NAME	125	Acute	n.d./n.d.	Implication of NO	-Sobrino et al. 2021b)
Cabernet wine lees	Dried liquid fraction	SHR+ Sirtinol	125	Acute	n.d./n.d.	Implication of SIRT1	
Cabernet wine lees	Dried liquid fraction	SHR+ Indomethacin	125	Acute	n.d./n.d.	Implication of PGI2	
Cabernet wine lees	Dried liquid fraction	SHR	125	Acute	48.0/42.6 mmHg	↑ NO, GSH ↓ MDA, ROS	(López-Fernández -Sobrino et al. 2021d)

Abreviatures: ACE (angiotensin-converting enzyme), Ang II (angiotensin II), BSO (buthionine sulfoximine), CAT (catalase), CHR (cafeteria diet-fed hypertensive rats), DOCA (desoxycortisone acetate), ERK-1/2 (extracellular signal-regulated kinases), eNOS (endothelial nitric oxide synthase), ET-1 (endothelin 1), GPx (gluthatione peroxidase), GSH (reduced glutathione), LA (left atrial functional capacities), L-NAME (Nw-nitro-L-arginine-methyl-ester), MDA (malondialdehyde), NIE (negative ionotropic effect), NO (nitric oxide), NOX4 (NADPH oxidase subunit 4), PGFα1 (prostaglandin f1 alpha), PGI2 (prostacyclin I2), PIE (positive ionotropic effect), RA (right atrial functional capacities), ROS (reactive oxygen species), SHR (spontaneously hypertensive rats), SIRT1 (sirtuin 1), SOD (superoxide dismutase), TBARS (thiobarbituric acid reactive substances)
n.d.: non-determined
(-): A dash appears when the specific blood pressure drop is not specified.

739 Table 2. Antihypertensive effects of grapes, wine or winemaking by-products in humans and molecular mechanisms involved in their
740 bioactivity.

Model	Grape source	Extraction condition/ Manufacturer	Dose	Administration duration	ΔSBP/DBP	Action mechanism	Ref.
Men with high cardiovascular risk	Dealcoholized red wine	none	272 mL/day	4 weeks	5.8/2.3 mmHg	NO	(Chiva-Blanch et al. 2012)
Healthy men	Red wine	none	8.1± 0.9 dL	Acute	n.d.	↓ ET-1	(Kiviniemi et al. 2010)
Healthy men	Dealcoholized red wine	none	8.1± 0.9 dL	Acute	n.d.	↓ ET-1	
Adults with high cardiovascular risk	Pomace	n.e	2 g/day	6 weeks	16.75 % in MAP	n.d	(Taladrid et al. 2022b)
Men with metabolic syndrome	Pomace	none	20 g/day	16 weeks	4.3/5.3 mmHg	n.d.	(Urquiaga et al. 2015)
Adults with mild HTN	Seed	Enovita®; Indena	150 mg+150 mg (breakfast and dinner)	16 weeks	4.6/3.2 mm Hg (only in men)	n.d	(Schön et al. 2021b)
Pre-hypertensive middle-aged Japanese men and women	Seed	Gravinol®; Kikkoman Biochemifa Company	400 mg/day	12 weeks	13.1/6.5 mmHg	n.d.	(Odai et al. 2019)
Pre-hypertensive	Seed	MegaNatural® BP; Polyphenolics	300 mg + exercise	Acute	↓SBP/↓DBP/ ↓MAP	↑endothelial function (FMD)	(Kim et al. 2018)

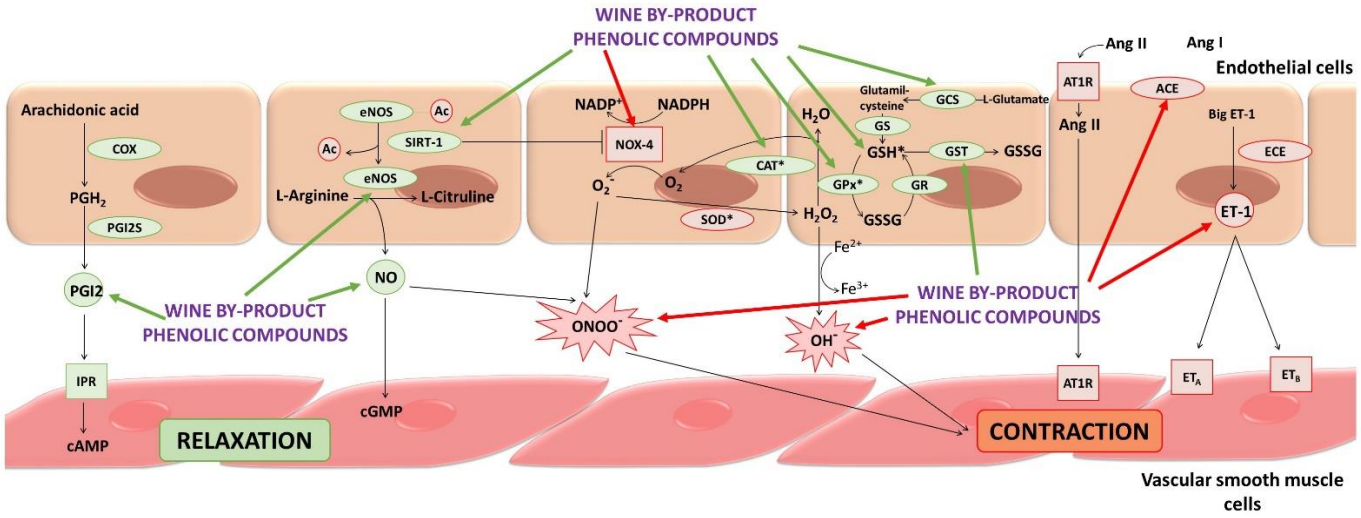
and sedentary men							
Pre-hypertensive men and women	Seed	MegaNatural® BP; Polyphenolics	300 mg/day	6 weeks	9/4 mmHg	n.d.	(Park et al. 2016)
Middle-aged women	Seed	Gravinol®; Kikkoman Biochemifa Company	100 or 200 mg/day	8 weeks	↓SBP/↓DBP	n.d	(Terauchi et al. 2014)
Adult female with pre-HTN or stage 1 HTN	Seed	Enovita®; Indena	300 mg/day + management plant	16 weeks	29/6.6 mmHg	n.d	(Belcaro et al. 2013)
Adult female with pre-HTN or stage 1 HTN	Seed	Enovita®; Indena	150 mg/day + management plant	16 weeks	18/3.6 mmHg	n.d	(Belcaro et al. 2013)
Adults with pre-HTN	Seed	MegaNatural® BP; Polyphenolics	300 mg/day	8 weeks	8/5 mmHg	n.d	(Kappagoda 2012)
Adults with metabolic syndrome	Seed	MegaNatural® BP; Polyphenolics	150 mg/day	4 weeks	11/6 mmHg	n.d.	(Sivaprakasapillai et al. 2009)
Adults with metabolic syndrome	Seed	MegaNatural® BP; Polyphenolics	300 mg/day	4 weeks	11/7 mmHg	n.d.	(Sivaprakasapillai et al. 2009)
Hypertensive patients	Grape polyphenols	n.e	150-1400 mg/day	2-16 weeks	1.48 /- mmHg	↑ NO	(Li et al. 2015)
Adults men with metabolic syndrome	Grape polyphenols	n.e	40 g/day	30 days	6/0 mmHg	↑ NO	(Barona et al. 2012)

Adults with different pathologies (Meta-analysis; 28 studies)	Grape products and by-product-derived extracts	-	-	chronic	3.4/1.69	n.d	(Asbaghi et al. 2021b)

Abreviatures: BP (blood pressure), ET-1 (endothelin 1), FMD (flow-mediated dilatation), HTN (hypertension), MAP (mean blood pressure), NO (nitric oxide), n.d **(**non-determined), n.e (non-explained)

(-): A dash appears when the specific blood pressure drop or the extraction method are not specified.

Figure 1. Effects of phenolic compounds from winery by-products on endothelium.



Green and red arrows represent stimulatory/upregulation or inhibitory/downregulation effects of winery by-product phenolic compounds on different endothelial function components. Vasorelaxant enzymes and molecules are represented in green and vasoconstrictor enzymes and molecules in red. ACE: angiotensin-converting enzyme; Ang: angiotensin; AT1R: Angiotensin type-1 receptor; cAMP: cyclic adenosine monophosphate; CAT: catalase, cGMP: cyclic guanosine monophosphate; COX: cyclooxygenase; ECE: endothelin-converting enzyme; ET-1: endothelin 1; ET_A: endothelin receptor A; ET_B: endothelin receptor B; eNOS: endothelial nitric oxide synthase; GCS: gamma-glutamylcysteine synthetase, GST: glutathione S-transferase, GR: glutathione reductase, GSH: reduced glutathione, GSSG: glutathione disulfide, GPx: glutathione peroxidase, IPR: prostaglandin receptor, L-Arg: L-arginine; NADPH: nicotinamide adenine dinucleotide phosphate; NOX-4: nicotinamide adenine dinucleotide phosphate-oxidase 4; NO: nitric oxide; PGI2: prostaglandin I2 or prostacyclin; PGI2S: Prostaglandin I_2 synthase; ROS: reactive oxygen species;

SIRT-1: Sirtuin 1; SOD: superoxide dismutase. * indicates that the effects of winery by-products on the different enzymes have not been directly evidenced in endothelial cells.