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Wine pomace seasoning attenuates hyperglycaemia-induced endothelial dysfunction and oxidative damage in endothelial cells

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ABSTRACT

Dietary antioxidants such as phenolic phytochemicals may prevent/improve endothelial function impairment observed in cardiovascular diseases. This study investigates the specific protective effects of a vegetal seasoning obtained from seedless wine pomace against hyperglycaemia-induced oxidative damage and dysfunction in human umbilical vein endothelial cells EA.hy926. Non-cytotoxic doses of the bioactive compounds obtained following *in vitro* digestion of the seasoning were used as treatments. Digested compounds, especially colonic bacterial metabolites, restored a more balanced redox environment, prevented lipid peroxidation and cell membrane damage, ameliorated protein oxidation, and improved the balance between endothelial reactive oxygen species and nitric oxide production in hyperglycaemic cells. Reduction of angiotensin I-converting enzyme activity and gene modulation of superoxide dismutase, haem oxygenase-1, β-nicotinamide adenine dinucleotide phosphate oxidase-4, and endothelial nitric oxide synthase are proposed as the mechanisms underlying this protection. These results support the potential benefits of functional wine pomace seasonings in vascular complications associated with oxidative stress.

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Abbreviations: **ABTS**, 2,2′-azinobis 3-ethylbenzothiazoline-6-sulphonic acid; **ACE**, angiotensin I-converting enzyme; **Ang**, angiotensin; **AREs**, antioxidant responsive elements; **BCF**, potentially bioavailable fraction obtained after simulated colonic fermentation; **BGID**, potentially bioavailable fraction obtained after simulated gastrointestinal digestion; **CGs**, carbonyl groups; **eNOS**, endothelial nitric oxide synthase; **FC**, Folin–Ciocalteu; **GAE**, gallic acid equivalents; **GL**, glucose; **GSH**, glutathione reduced; **GSSG**, glutathione oxidised; **HO**, haem oxygenase; **HUVECs**, human umbilical vein endothelial cells; **LDH**, lactate dehydrogenase; **MDA**, malondialdehyde; **mDP**, mean degree of polymerisation; **NAD(P)H**, β-nicotinamide adenine dinucleotide phosphate; **NF-κB**, nuclear factor-kappa B; **NO**, nitric oxide; **NOX**, NAD(P)H oxidase; **Nrf2**, nuclear factor-erythroid 2-related factor 2; **Q-**, QUENCHER; **RAAS**, renin-angiotensin aldosterone system; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TE**, Trolox equivalents

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1. Introduction

Oxidative stress plays a critical role in the pathogenesis and development of cardiovascular disorders, with a growing body of evidence indicating that impaired endothelial function is a common cause and/or consequence of diverse risk factors relating to cardiovascular diseases such as hypertension, diabetes mellitus, and hypercholesterolemia (Versari, Daghini, Virdis, Ghiadoni, & Taddei, 2009). An increased production of reactive oxygen species (ROS) and the subsequent decrease in vascular bioavailability of nitric oxide (NO) have long been proposed as one of many complex and multifactorial causes that contribute to endothelial dysfunction (Higashi, Noma, Yoshizumi, & Kihara, 2009).

The most relevant sources of ROS in vascular cells, mainly superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are NAD(P)H oxidase (NOX), uncoupled endothelial NO synthase (eNOS), xanthine oxidase, and the enzymes of the mitochondrial electron transport chain (Pennathur & Heinecke, 2007). The rapid reaction of $O_2^{\cdot-}$ with NO leads to an excessive formation of peroxynitrite ($ONOO^-$), a reactive nitrogen species that may act as a vasoconstrictor, and most importantly, as a cytotoxic molecule, causing direct structural damage to proteins, lipids, and DNA, as well as further ROS generation (Münzel, Gori, Bruno, & Taddei, 2010).

A reduction in NO production has also been described as one of the mechanisms leading to the lower bioavailability of NO observed in the pathogenesis of several vascular complications. The main causes reported to underlie this decreased formation of NO are reduced eNOS mRNA levels and/or stability, and decreased synthesis, activation, and/or activity of eNOS enzymes (Schmitt & Dirsch, 2009; Versari et al., 2009).

Another factor implicated in the development of oxidative stress and injury in the vascular system is the overactivation of the renin-angiotensin aldosterone system (RAAS). Angiotensin I-converting enzyme (ACE) is crucial in the regulation of RAAS, as it cleaves angiotensin I (Ang I) to produce the potent vasoconstrictor angiotensin II (Ang II) (Hsueh & Wyne, 2011; McFarlane, Kumar, & Sowers, 2003). The major mechanisms by which Ang II causes vascular damage include ROS generation and the stimulation of redox-dependent signalling pathways in endothelial and other vascular cells (Virdis, Duranti, & Taddei, 2011). Therefore, RAAS is also involved in the control of NO bioavailability in vasculature. However, the inhibition of ACE activity has been suggested as a promising therapeutic approach for the prevention and treatment of several cardiovascular diseases and associated complications (Balasuriya & Rupasinghe, 2011; McFarlane et al., 2003).

Living organisms have developed several effective mechanisms to protect themselves from the detrimental effects of ROS. The endogenous antioxidant-defence system includes enzymes such as superoxide dismutases (SOD), catalase, glutathione peroxidases, haem oxygenases (HO), and others, as well as non-enzymatic antioxidants such as glutathione (GSH) (Lee, Margaritis, Channon, & Antoniades, 2012).

In addition, several exogenous interventions using antioxidant dietary compounds have been proposed to prevent endothelial dysfunction and to restore the redox status in the vascular microenvironment, with certain phenolic compounds being highlighted among the most promising natural antioxidants with beneficial cardiovascular effects (Stoclet et al., 2004). In this regard, a number of studies have been initiated, dealing with the antioxidant, hypotensive, and vascular health-promoting effects of phenolic compounds in grapes, wine, and wine pomace. The antioxidant capacity of these phytochemicals is not simply related to their direct ROS scavenging ability, but also to the inhibition of enzymatic sources of oxidative stress, such as NOX and ACE, as well as the stimulation of endogenous antioxidant enzymes (Eriz, Sanhueza, Roeckel, & Fernández, 2011; Münzel et al., 2010; Nijveldt et al., 2001).

In relation to wine pomace, this winemaking by-product has traditionally been treated as a winery residue, but there is growing industrial and scientific interest nowadays in alternative uses for its value adding properties (Dwyer, Hosseinian, & Rod, 2014). In this context, new powdered seasonings obtained from wine pomace have recently been developed (García-Lomillo, González-Sanjosé, Del Pino-García, Rivero-Pérez, & Muñiz, 2014). These seasonings represent an innovative strategy to mitigate problems related to the disposal of wine pomace in wineries, while taking advantage of the bioactive phytochemicals, including phenolic antioxidants, remaining in such by-products. Taking into account that wine pomace seasonings have demonstrated significant antioxidant properties *in vitro* (Del Pino-García, García-Lomillo, Rivero-Pérez, González-Sanjosé, & Muñiz, 2015), their use as natural additives or ingredients in functional foods is expected to enhance exogenous antioxidant dietary pool and contribute to maintaining/restoring a healthy internal redox status.

However, one of the principal topics concerning the beneficial effects of phenolic compounds is their bioavailability, which is dependent upon their digestive stability, their bioaccessibility (release from the food matrix), the efficiency of their trans-epithelial passage, and their further metabolism in enteric and hepatic tissue, as well as by gut microbiota (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Scalbert & Williamson, 2000; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Therefore, despite the limitations of simulated digestion systems (Alminger et al., 2014), the combination of *in vitro* digestion protocols with cell assays has recently been proposed as a suitable approach to determine the antioxidant activity of phenolic compounds once they have been ingested (Huang, Sun, Lou, & Ye, 2014).

A frequently used model to investigate endothelial function impairment and associated pro-oxidative environment *ex vivo* is the incubation of vascular cells such as human umbilical vein endothelial cells (HUVECs) under high glucose concentrations (Inoguchi et al., 2000; Koziel, Woyda-Ploszczyca, Kicinska, & Jarmuszkiewicz, 2012; Patel, Chen, Das, & Kavdia, 2013; Zhou, Xie, Zhou, & Li, 2012). The rationale behind this model lies in the significant oxidative stress observed in the vascular system of diabetic patients, which may lead to important vascular complications and is well-known to be caused, at least in part, by the hyperglycaemic conditions that characterise diabetes (Fatehi-Hassanabad, Chan, & Furman, 2010; Hadi & Suwaidi, 2007).

In view of the above, this paper is focused on the potential protective effects against endothelial dysfunction and oxidative damage in the vasculature of new natural food ingredients, trying to bring together the results of fundamental