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## Accepted Manuscript






ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

View Article Online
DOI: 10.1039/D6FO01211F

PAPER

# Moderate consumption of a polyphenolic-enriched wine attenuates alcohol-associated alterations in hepatic biomarkers in overweight subjects: a randomized clinical trial

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Moderate red wine consumption has been associated with cardioprotective effects, which have been partly attributed to its polyphenolic content. However, human intervention studies remain limited, particularly regarding polyphenol-enriched wines with reduced alcohol content. In this study, we have formulated and characterized a non-fermented grape skins polyphenol-enriched (GSPE) red wine reduced in alcohol, and its effects were evaluated in a randomized, double-blind intervention comparing GSPE wine with a reduced-alcohol control red wine on metabolic and antioxidant-related biomarkers in overweight adults or individuals presenting components of the metabolic syndrome. After a three-week alcohol washout period, participants were randomly assigned to consume either GSPE wine (n = 21) or control wine (n = 22) daily for 5–6 weeks. Anthropometric parameters, blood pressure, antioxidant levels, and biochemical markers were assessed at baseline and at the end of the intervention. Consumption of the GSPE wine resulted in a significant reduction ($p < 0.05$) in serum alanine aminotransferase levels compared with baseline and relative to the control group, with no adverse changes in other hepatic parameters. In contrast, control wine showed a significant increase in plasma uric acid levels and total antioxidant activity, together with a slight but significant decrease in albumin concentration ($p = 0.03$). These parameters were not significantly altered following GSPE wine intake. Taken together, these findings suggest that polyphenol-enriched wine may attenuate alcohol-associated alterations in hepatic biomarkers.





## 1 Introduction

Numerous epidemiological studies have associated moderate and regular wine consumption with lower mortality and morbidity from chronic diseases, primarily cardiovascular disease (CVD), the current leading cause of death worldwide 1. In contrast, excessive or binge alcohol consumption is clearly associated with increased morbidity and mortality, as well as with work-related and traffic accidents.[1, 2]

Although several human clinical intervention studies have investigated the effects of red wine consumption, most have been short- to medium-term, involved relatively small sample sizes, and focused primarily on biomarker-based outcomes rather than long-term clinical events,[3] Nevertheless, these studies consistently report beneficial effects of moderate red wine intake on plasma antioxidant capacity, lipid profile, and coagulation parameters, which may partly explain the reduced risk of CVD and overall mortality observed in moderate drinkers.[1, 4, 5]

In general, chronic pathologies, including obstructive cardiovascular and cerebrovascular disease, diabetes, Alzheimer's disease, and cancer, are associated with oxidative damage to lipids, proteins, and DNA, and a decrease in the endogenous antioxidant defenses.[6, 7] In fact, CVD is primarily due to atherosclerosis, a degenerative process of the arteries triggered by oxidative stress and a chronic inflammatory state. [8-12]. The biological effects of red wine on human health have traditionally been attributed to both ethanol content (associated with increased HDL-cholesterol levels and decreased blood fibrinogen)[13] and polyphenolic fraction, which exhibits antioxidant and anti-inflammatory effects.[5] Red wine contains a wide variety of polyphenols, including phenolic acids (gallic acid), flavonoids (catechin, epicatechin, quercetin, anthocyanins, and procyanidins), and stilbenes such as resveratrol (3,4',5-trihydroxystilbene), among others. [1, 14, 15].

Despite these potential benefits, ethanol intake can also promote oxidative stress through several metabolic pathways, constituting a major mechanism underlying alcohol-induced toxicity.[16] The liver is the main site of ethanol metabolism and a major target organ of ethanol-induced injury.[17] While moderate alcohol intake is mainly metabolized via alcohol dehydrogenase,

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higher or chronic consumption induces the cytochrome P450 system, particularly CYP2E1, a major source of reactive oxygen species.[17, 18] This oxidative burden may contribute to hepatic steatosis and other metabolic disturbances.[18] Alcohol-induced oxidative stress may be modulated by endogenous antioxidants (e.g., glutathione) and dietary antioxidants (e.g., flavonoids), potentially limiting associated hepatic damage.

Polyphenols present in red wine have been shown to reduce oxidative damage to lipids, particularly by inhibiting LDL oxidation and subsequent arterial deposition, and have also been associated with improved insulin sensitivity in individuals with type 2 diabetes or high cardiovascular risk.[5, 19] At the molecular level, these compounds modulate key signaling pathways involved in redox homeostasis and inflammation, including activation of the Nrf2/ARE pathway, leading to increased glutathione synthesis, upregulation of antioxidant enzymes, and inhibition of NF-κB and MAPK signaling, thereby attenuating inflammatory responses.[20-23]. Consistent with these mechanisms, clinical studies have reported reduced oxidative damage to lipids (measured as malondialdehyde), proteins, and DNA, increased plasma antioxidant capacity, and improved cardiometabolic markers following red wine consumption.[4, 5, 24-27] Interestingly, studies using dealcoholized red wine have also demonstrated reductions in blood pressure and insulin resistance, highlighting the contribution of the polyphenolic fraction independently of ethanol.[5, 27]

In this context, reducing the alcohol content of red wine is a topic of growing interest, driven on the one hand by consumer health concerns related to the ethanol-induced oxidative damage and other complications[16], and on the other hand by challenges faced by the wine industry, particularly the increase in grape must sugar content in some regions due to climate change, which consequently leads to higher alcohol by volume in wines[28]. The effects of dealcoholizing on the polyphenolic content of red wines have shown to depend on the type of wine and techniques employed, with reports showing maintained or even increased concentrations of total phenolics and anthocyanins in reduced-alcohol wines.[29] Moreover, grape skins, a by-product of grape processing, are rich sources of polyphenolic compounds, including flavonoids, stilbenes, and phenolic acids.[30] It has been shown that phenolic composition of grape skins exhibits different phenolic profiles when compared with seeds, with skins being mainly associated with anthocyanins.[31] Phenolic compounds present in red wine and grape pomace have attracted considerable interest due to their antioxidant, anti-inflammatory, antihypertensive, anticancer, and antibacterial activities.[30, 32]

However, to date, no human studies have specifically evaluated the health effects of red wine with reduced alcohol content supplemented with non-fermented grape skin–derived polyphenols. Therefore, the aim of this study was to formulate a red wine with reduced alcohol content enriched with non-fermented grape skin–derived polyphenols and to evaluate, through a clinical trial in men and women, the effects of moderate consumption of this polyphenol-enriched, low-alcohol, low-calorie wine compared with a control wine (low alcohol, traditional polyphenol content) on markers of metabolic syndrome, antioxidant status, and oxidative damage.

# 2 Materials and methods

### 2.1 Chemicals

Methanol (HPLC grade, 99.9% purity), water (LC-MS grade, >99.9% purity), acetonitrile (HPLC grade, 99.9% purity), formic acid (analytical grade, >98.0), isopropanol, Folin-Ciocalteu reagent, were purchased from Merck Millipore (Darmstadt, Germany). All the polyphenolic standards except caftaric acid and epicatechin gallate were purchased from Extrasynthese (France). Caftaric acid (≥98% purity) and epicatechin gallate (≥98% purity) were purchased from Cayman chemical company (MI, USA).

### 2.2 Grape skin polyphenols extraction

Grape pomace (seeds and skins) prior to fermentation from the Malbec variety was obtained as a by-product of the winemaking process at the Viña Concha y Toro production plant (Pencahue, Chile). Skins were separated from seeds using a sieving mesh. The grape pomace skins were then ground at 2500 rpm for 15 min and extracted using an ethanol: water (3:2) mixture for 4 h. The resulting extracts were subsequently dried by spray drying and stored for subsequent enrichment of low-alcohol wine. The phenolic composition of the grape skin polyphenol concentrate is shown in Table S1.

### 2.3 Dealcoholized wine production and generation of non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine

Dealcoholization of the control red wine and the GSPE wine was performed by reverse osmosis (Dimerco–Memstar, model R08-4). Alcohol content was monitored using an Alcolyzer Wine device (Anton Paar, Graz, Austria). Subsequently, the GSPE wine was enriched with polyphenol-rich extracts obtained from grape skin pomace, which were fully dissolved in the wine matrix. Finally, both the control red wine and the GSPE wine were bottled for chemical characterization and use in the intervention study.

### 2.4 Characterization of control and GSPE wines

The control wine and the non-fermented skin-polyphenol-enriched (GSPE) wine were characterized in terms of alcohol content, total polyphenols, total anthocyanins, antioxidant capacity, and phenolic composition. Alcohol degree was determined in previously degassed samples using an Alcolyzer Wine device (Anton Paar GmbH, Graz, Austria), according to the manufacturer's instructions. Calorie content was calculated using Atwater factors, and uncertainty in caloric values was estimated by error propagation from the standard deviation of alcohol content, assuming constant ethanol density and an energy yield of 7 kcal $g^{-1}$. Total polyphenol concentration was determined by the Folin–Ciocalteu method[33] and expressed as mg of gallic acid equivalents (GAE) per mL of sample. Total monomeric anthocyanins were quantified using AOAC Official Method 2005.02 and expressed as mg of cyanidin-3-glucoside

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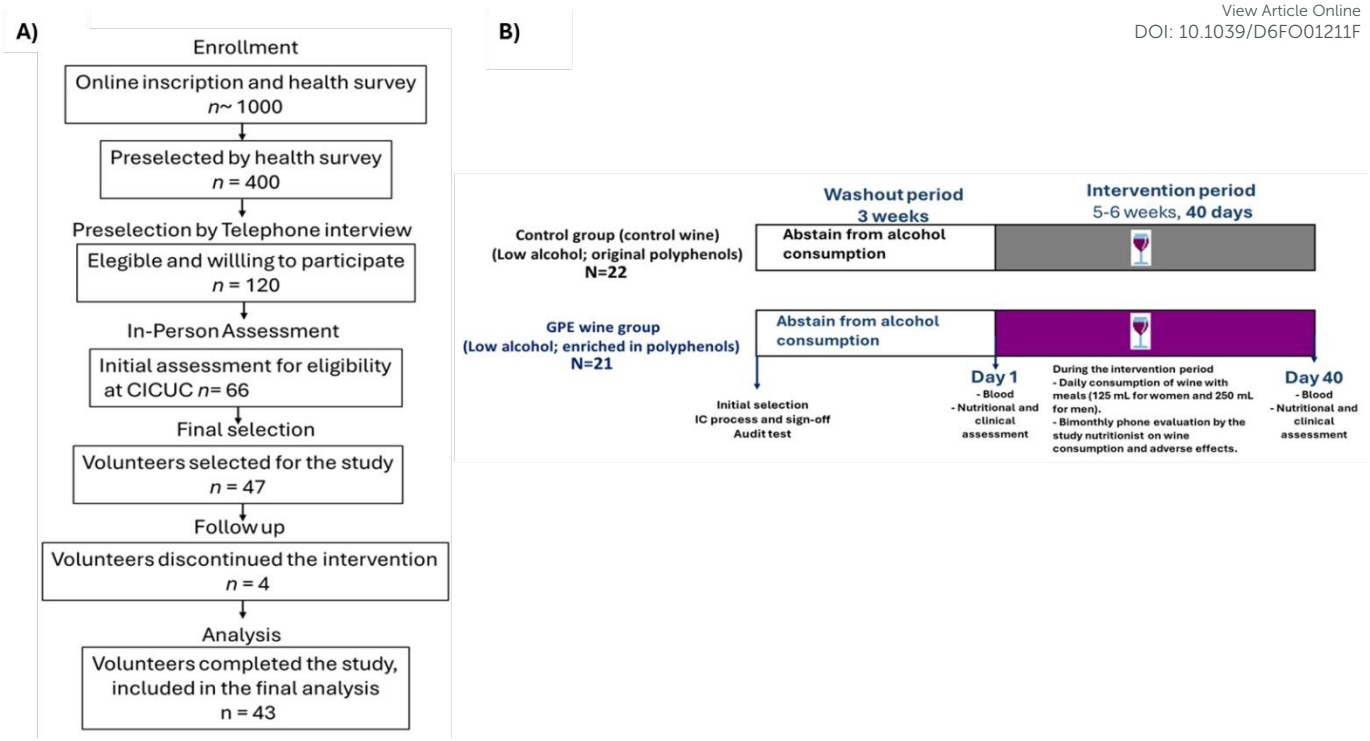


**Fig. 1** CONSORT-based study flow diagram (A) and study design of the double-blind, randomized nutritional intervention trial (B). Forty-seven subjects were randomized to the control wine or non-fermented grape pomace skin polyphenol-enriched (GSPE) wine groups; 43 completed the intervention. Following a 3-week washout period, participants consumed daily 125 mL (women) or 250 mL (men) of control or GSPE wine for 40 days. Blood samples and nutritional and clinical assessments were performed at baseline and at the end of the intervention.

equivalents per mL.[34] Antioxidant capacity was evaluated by the Oxygen Radical Absorbance Capacity (ORAC) assay following the method described by Cao et al. [35], and results were expressed as µmol Trolox equivalents (TE) per mL. The phenolic composition of both wines was analyzed by HPLC-DAD-FL as described by Ferreyra et al. [36], using external calibration curves for Delphinidin-3-*O*-glucoside, Cyanidin-3-*O*-glucoside, Petunidin-3-*O*-glucoside, Peonidin-3-*O*-glucoside, Peonidin 3-*O*-(6-acetyl-glucoside), Malvidin 3-(6-acetylglucoside), Peonidin 3-*O*-(6-*p*-coumaroyl-glucoside), Malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside, Catechin, Epicatechin, Epicatechin gallate, Procyanidin B1, Procyanidin B2, Procyanidin B3, Gallic acid, Ellagic acid, *p*-Coumaric acid, Caffeic acid, Caftaric acid, Quercetin, Myricetin, Kaempferol, Myricetin-3-*O*-galactoside, Myricetin-3-*O*-glucoside, Quercetin-3-*O*-glucoside, Rutin (quercetin-3-*O*-rutinoside), Hyperoside (quercetin-3-*O*-galactoside), Syringetin-3-*O*-galactoside, Syringetin-3-*O*-glucoside, Resveratrol, Polydatin (resveratrol-3-*O*-glucoside), and Viniferin.

### 2.5 Participants

Adult volunteers (men and women) who were regular red wine consumers were recruited through the online platforms of Viña Concha y Toro. Interested individuals completed an online health survey, including age, place of residence, and patterns of alcohol consumption in general and red wine consumption in particular. Individuals aged 30–65 years, residing in the Metropolitan Region, and reporting red wine consumption ≥3 times per week were considered eligible for preselection. Approximately 1,000 individuals completed the online survey, of whom 400 met the preselection criteria (Fig. 1). These individuals were subsequently contacted by telephone to explain the study procedures and to collect additional clinical information related to exclusion criteria, resulting in 120 eligible and willing participants. Of these, 66 attended an in-person visit at the Clinical Research Center of the Pontificia Universidad Católica de Chile for eligibility assessment. During this visit, participants provided written informed consent and underwent a comprehensive baseline evaluation, including completion of a clinical record with family medical history and cardiovascular risk factors, anthropometric measurements, blood pressure assessment, and evaluation of alcohol consumption patterns using the Alcohol Use Disorders Identification Test (AUDIT). In addition, fasting blood samples were collected for hematological analysis, lipid profile, biochemical profile, liver function tests, and measurement of thyroid-stimulating hormone (TSH). Following this eligibility assessment, 47 volunteers were finally selected and enrolled in the study. During the intervention period, 4 participants discontinued the study, and 43 volunteers completed the intervention and were

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included in the final analysis (Fig. 1). Among the 43 participants who completed the study, 28 subjects (65.1%) strictly adhered to the protocol (no missed wine consumption and no intake of other alcoholic beverages). Protocol deviation was reported in 7 participants (16.3%) who missed wine consumption for no more than 2 days, and in 8 participants (18.6%) who reported consuming another alcoholic beverage on up to 2 occasions during the intervention, mainly beer or sparkling wine in moderate amounts. No participant reported both missed wine consumption and intake of other alcoholic beverages.

The study protocol was approved by the Health Sciences Scientific Ethics Committee of the Pontificia Universidad Católica de Chile (approval no. 230502020). The trial was registered at the ISRCTN registry (ISRCTN10066959).

### 2.6 Inclusion and exclusion criteria

Inclusion criteria were as follows: voluntary agreement to participate in the study and provision of written informed consent; men and women aged 30–65 years; regular red wine consumption; an Alcohol Use Disorders Identification Test (AUDIT) score <8; and overweight status (body mass index (BMI) 25–35 kg $m^{-2}$) or the presence of at least one component of the metabolic syndrome. The presence of metabolic syndrome components was defined using the criteria proposed by the Adult Treatment Panel III of the US National Cholesterol Education Program[37] adapted for Chilean population[38], which are: (1) abdominal obesity as waist circumference ≥ 90 cm in men or ≥ 80 cm in women; (2) low levels of serum high-density lipoprotein (HDL) cholesterol, < 40 mg $dL^{-1}$ in men or < 50 mg $dL^{-1}$ in women; (3) hypertriglyceridemia (triglycerides ≥150 mg/dL); (4) elevated blood pressure (≥130/85 mmHg); and (5) fasting plasma glucose levels of ≥100 mg $dL^{-1}$.

Exclusion criteria included obesity (BMI >35 kg $m^{-2}$); diagnosis of diabetes mellitus; severe dyslipidemia (triglycerides >500 mg $dL^{-1}$ and/or total cholesterol >300 mg $dL^{-1}$); active liver disease; rheumatologic or other chronic conditions that could interfere with study measurements; history of clinically overt cardiovascular events; use of antioxidant supplements or medications known to affect lipid metabolism; use of psychoactive drugs; and potential problematic alcohol consumption, defined as an AUDIT score ≥8.

Participants who were consuming antioxidant supplements but were willing to discontinue their use prior to being enrolled were allowed to participate. In addition, individuals with pharmacologically controlled hypertension or hypercholesterolemia treated with lipid-lowering agents for at least 1 year were also eligible.

### 2.7 Study design

This study was designed as a randomized, double-blind intervention trial. The sample size was determined to detect differences in the plasma TAR index at the end of the intervention. Considering a 5% alpha error and 85% statistical power and an expected 20% increase in the TAR index, a minimum of 20 participants per group was required (effect size = 0.873). Prior to the consumption of the experimental and control wines, participants underwent a three-week alcohol washout period, which was considered sufficient for the lipid profile to return to baseline levels, as previously reported[39,40]. After completion of the washout period, participants were recalled to the Clinical Research Center of the Pontificia Universidad Católica de Chile (CICUC) to initiate the intervention.

Participants were randomly assigned, using a computer-generated randomization program, to one of two parallel groups, with participants, healthcare professionals, and laboratory staff blinded to group allocation. One group received the GSPE wine, enriched in polyphenols and reduced in alcohol content, whereas the other group received the control wine, characterized by conventional polyphenol content and reduced alcohol content. Biological samples were labelled using a study-specific code followed by the participant identification number and sampling time point, ensuring that laboratory personnel remained blinded to treatment allocation throughout the analyses.

Participants were instructed to consume wine daily with the main meals (lunch and dinner), at a dose of 125 mL (one glass) for women and 250 mL (two glasses) for men. The intervention lasted approximately 5–6 weeks (mean duration of 41 days), depending on participants' availability to attend the final evaluation and sample collection visit at the CICUC. To support adherence, participants received a kit including written consumption instructions, a consumption logbook, a calibrated glass corresponding to 125 mL, and a vacuum pump for proper wine bottle storage. The wine was delivered directly to participants' homes. Compliance with the intervention was monitored through regular follow-up telephone calls by a dietitian, who was also available to address participants' questions or concerns.

Throughout the intervention period, participants were instructed to abstain from consuming any alcoholic beverages other than the study wine. No changes to habitual diet or lifestyle were required, except for the prescribed wine consumption. At baseline and at the end of the intervention, participants underwent a medical and nutritional evaluation, including assessment of clinical history and cardiovascular risk factors with emphasis on metabolic syndrome components. Anthropometric measurements (body weight, height, waist circumference, and BMI) and blood pressure were recorded, and fasting blood samples were collected for biochemical analyses.

Overall dietary intake was assessed at both time points using a validated self-reported questionnaire consisting of 14 items to evaluate adherence to the Mediterranean diet adapted for the Chilean population (Chilean-MDI). The score ranged from 0 (minimal adherence) to 14 (maximal adherence). At the end of the study, participants' perception of wine consumption was assessed by telephone interview

### 2.8 Anthropometric and blood pressure measurements

Height and weight were recorded at the start and end of the intervention period. Systolic and diastolic blood pressures were measured on the left arm at heart level after at least 5 min of resting in a sitting position, using an Automatic upper arm blood

pressure monitor HEM-7120 (Omron, Kyoto, Japan). Two readings, separated by at least 1 min, were taken, and the mean value was calculated and recorded. If there was more than a 5 mm Hg difference between the first and second readings, an additional reading was obtained and used to calculate the recorded mean value.[41]

### 2.9 Mediterranean diet score

Overall food intake was evaluated using a self-reported questionnaire with fourteen items that measured adherence to the Mediterranean diet in Chile, the Chilean-MDI.[42] This scale was designed based on traditional food consumption habits in Mediterranean countries, with selective modifications to incorporate Chilean dietary habits. Scores ranged from 0 (minimal adherence) to 14 (maximal adherence).

### 2.10 Biochemical procedures

Venous blood samples were taken after a 12 h fasting period and collected in heparin, citrate, EDTA, and anticoagulant-free BD Vacutainer® tubes. Glucose, albumin, uric acid, bilirubin, creatinine, calcium, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and alkaline phosphatase were measured in plasma using a spectrophotometer autoanalyzer (Hitachi 917; Roche Diagnostics®, Branchburg, NJ, USA) with reagent kits purchased from the manufacturer. Insulin was measured by electrochemiluminescence immunoassay (ECLIA) (Roche Diagnostics®, Mannheim, Germany).

The total plasma antioxidant reactivity (TAR) was evaluated from luminol-enhanced chemiluminescence measurements (Bio-Orbit 1250, Turku, Finland).[43]

Total phenolic compounds in urine were determined by the Folin–Ciocalteu method with removal of protein interference.[44] Urine samples were deproteinized using a solid-phase extraction using Oasis® MAX 96-well plate cartridges and the rapid Folin–Ciocalteu protocol described by Medina-Remón et al.[45]

To determine vitamin C levels, blood samples kept on ice were analyzed the same day that blood was drawn. L-Ascorbic acid was determined by spectrophotometry using a multi-detection microplate reader, Synergy HT (BIO-TEK, Montpelier, VT, USA) .[46] Advanced oxidation protein products (AOPPs) were determined in the plasma using the method described by Witko-Sarsat et al.[47, 48] Oxidized low-density lipoprotein (oxLDL) was determined by the oxLDL/MDA Adduct Enzyme-Linked ImmunoSorbent Assay (ELISA) Kit (Immundiagnostik, AG, Bensheim, Germany). For protein oxidation products and advanced glycation end products (AGEs), plasma protein concentration was determined using the bicinchoninic acid assay (Thermo Scientific, Rockford, IL, USA), with bovine serum albumin as the standard protein. Samples were adjusted to a final protein concentration of 1.7 mg $mL^{-1}$ in the cuvette before analysis. Intrinsic fluorescence measurements were made using a Perkin-Elmer LS 55 spectrofluorometer (Perkin-Elmer Ltd., Waltham, MA, USA). The following excitation/emission were measured: tryptophan (Ex: 295/Em: 360 nm), N-formylkynurenine (N-formylKyn; Ex: 325/Em: 434 nm), advanced glycation end products 1 (AGEs 1; Ex: 325/Em: 440 nm), dityrosine (DiTyr; Ex: 330/Em: 415 nm), kynurenine (Kyn; Ex: 365/Em: 480 nm), and advanced glycation end products 2 (AGEs 2; Ex: 389/Em: 443 nm).[49]

### 2.11 Statistical analysis

For *in vitro* analyses, statistical differences between the mean values of the GSPE wine and control groups were determined by one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test using the software SPSS 14.0 for Windows (IBM, Armonk, NY, USA). All *in vitro* experiments were performed in triplicate, and statistical significance was set at $p < 0.05$. For the dietary intervention, sample size calculation was performed using G*Power software (version 3.1.9.4), as described in the Study design section. Continuous variables were expressed as mean ± standard deviation (SD). Continuous outcomes were analysed using linear mixed-effects models adjusted for age and sex, including subject as a random effect and time as repeated measures. Group, time, and group x time interaction terms were included in the models. Categorical variables were analysed using the chi-square test. Baseline comparisons between groups were assessed using Student's t-test or chi-square test as appropriate. Statistical significance was set at $p < 0.05$

## 3 Results and discussion

### 3.1 Formulation and characterization of polyphenol-enriched wine from grape pomace skin

Total phenolics, anthocyanins, and ORAC activity were determined across the 14 varieties of grape pomace skin (Table S2). A principal component analysis (PCA) was performed to select the variety of non-fermented grape pomace skin to produce a wine enriched in polyphenols with a high antioxidant activity.

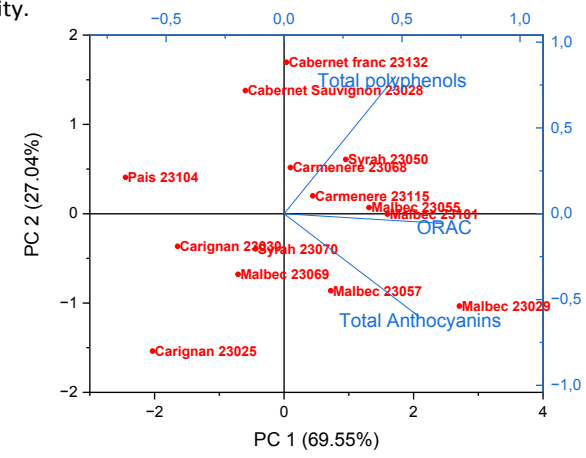


**Fig. 2** Principal component analysis (PCA) of total polyphenols, anthocyanins, and antioxidant activity (ORAC) in grape pomace skins from different grape cultivars.

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DOI: 10.1039/D5FO01311F

**Table 1** Characterization of alcohol content, calories, total polyphenols, anthocyanins, and ORAC antioxidant activity of a control wine and non-fermented grape pomace skin polyphenolic-enriched (GSPE) wine.

| Sample | Alcohol content (mL 100 mL$^{-1}$) | Calorie content (kcal 100 mL$^{-1}$) | Total polyphenols (mg GAE mL$^{-1}$) | Total anthocyanins (mg Eq C3G mL$^{-1}$) | ORAC (µmol TE mL$^{-1}$) |
|---|---|---|---|---|---|
| Malbec-based wine | 12.9 ± 0.00$^{a}$ | 71.2 ± 0.0$^{a}$ | 2.05 ± 0.07 | 0.19 ± 0.01 | 33.08 ± 2.86 |
| Control wine | 8.20 ± 0.02$^{b}$ | 45.3 ± 0.1$^{b}$ | 2.19 ± 0.09 | 0.20 ± 0.01 | 38.31 ± 0.42 |
| GSPE wine | 8.18 ± 0.03$^{b}$ | 45.2 ± 0.2$^{b}$ | 4.26 ± 0.21 | 0.82 ± 0.07 | 64.00 ± 2.87 |

Note: Results are expressed as mean ± SD (n = 10). Different letters indicate significant differences ($p < 0.05$) among samples according to one-way ANOVA followed by Tukey's post hoc test. Abbreviations: GAE: Gallic acid equivalents; C3G-eq: Cyanidin-3-glucoside equivalents; TE: Trolox equivalents (a water-soluble vitamin E analogue used as a standard for the calibration curve); ORAC (Oxygen Radical Absorbance Capacity).

The PC 1 explained a 69.6% of the total variance, representing a global antioxidant capacity index for the samples analysed (Fig. 2). The antioxidant activity determined by means of the ORAC assay was the most representative descriptor of the PC1 (loading 0.970), while anthocyanins (loading 0.824) and total polyphenols (loading 0.683) contributed in a complementary manner to the differentiation of the samples (Fig.2). Based on the component scores, the variety with the highest value, namely Malbec 23029, was identified as the non-fermented grape pomace skin with the greatest overall antioxidant capacity and was therefore selected among the 14 varieties of grape pomace to perform solvent extractions to generate a non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine.

Table 1 shows a statistically significant decrease of approximately 36% in alcohol content in both the control (low-alcohol) wine and GSPE wine compared with the Malbec-based wine. This reduction was accompanied by a concomitant decrease in total caloric content (Table 1). Compared with the control wine, the GSPE wine exhibited increases of 94.5% in total polyphenols, 310% in total anthocyanins, and 67% in ORAC antioxidant activity (Table 1).

The phenolic composition of the control and GSPE wines is shown in Fig. 3. The main differences were observed in the levels of malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside, petunidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside, which were 9.9, 6.3, 5.8, and 4.8 fold higher in the GSPE wine than in the control wine, respectively. The total anthocyanin concentration determined by HPLC is consistent with the values reported in Table 1. In the GSPE wine, the concentration was 654.67 mg L$^{-1}$, approximately 4.7-fold higher than that observed in the control wine (138.79 mg L$^{-1}$).

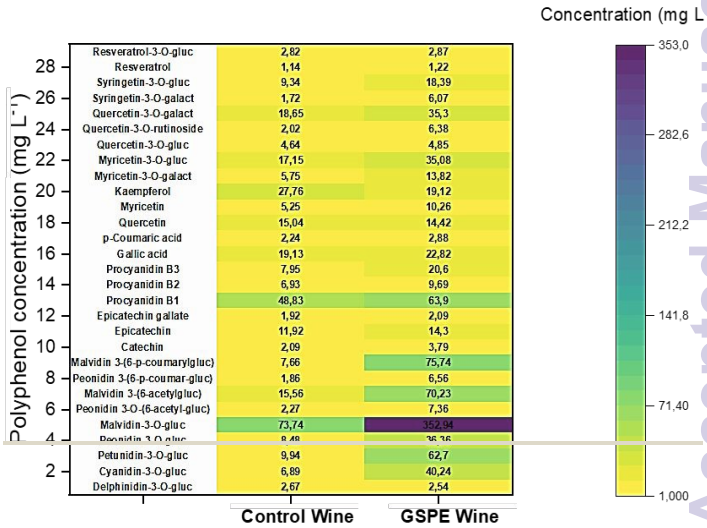


**Fig. 3** Heatmap of individual polyphenol concentrations (mg L$^{-1}$) in control wine and non-fermented grape pomace skin polyphenol-enriched (GSPE) wine. Each row represents a distinct phenolic compound, and colours indicate relative abundance according to the adjacent colour scale, with darker shades corresponding to higher concentrations. The data illustrate the differential impact of grape pomace extract (GSPE) supplementation on the polyphenolic profile of the wine.

### 3.2 Baseline characteristics

The effects of a daily consumption of control and GSPE wine in overweight subjects or those with at least one altered metabolic syndrome parameter were evaluated through a longitudinal, randomized, double-blind nutritional intervention study with parallel groups.

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DOI: 10.1039/D6FO01211F

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**Table 2** Baseline characteristics of the two groups of volunteers that completed the study.

| Variable | Control group (*n*= 22) | | | GSPE group (*n*= 21) | | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SD | Min | Max | Mean ± SD | Min | Max | p-val |
| Age (years) | 46.4 ± 8.3 | 31 | 61 | 46.9 ± 8.0 | 37 | 63 | ns |
| SBP (mmHg) | 121.4 ± 12.9 | 99 | 152 | 122.8 ± 15.8 | 102 | 161 | ns |
| DBP (mmHg) | 78.8 ± 8.1 | 65.5 | 98 | 82.3 ± 12.5 | 65.5 | 107.5 | ns |
| BMI (kg $m^{-2}$) | 26.9 ± 2.9 | 23.1 | 34.9 | 27.8 ± 2.9 | 22.2 | 34.9 | ns |
| Waist (cm) | 91.9 ± 10.1 | 77.5 | 116 | 93.3 ± 10.6 | 73.5 | 116 | ns |
| Gluc (mg $dL^{-1}$) | 89.2 ± 9.2 | 76 | 110 | 89.7 ± 6.6 | 80 | 110 | ns |
| AST (U $L^{-1}$) | 23.9 ± 4.9 | 17 | 33 | 26.3 ± 8.8 | 16 | 54 | ns |
| ALT (U $L^{-1}$) | 25.9 ± 10.9 | 12 | 51 | 30.9 ± 11.6 | 16 | 56 | ns |
| GGT (U $L^{-1}$) | 19.3 ± 10.6 | 8 | 46 | 21.5 ± 10.2 | 8 | 41 | ns |
| TC (mg $dL^{-1}$) | 196.5 ± 27.8 | 150 | 254 | 184.2 ± 32.3 | 118 | 266 | ns |
| TG (mg $dL^{-1}$) | 124.4 ± 59.2 | 62 | 291 | 101.2 ± 54.0 | 38 | 241 | ns |
| HDL-c (mg $dL^{-1}$) | 49.7 ± 15.9 | 32 | 86 | 54.6 ± 16.3 | 26 | 87 | ns |
| LDL-c (mg $dL^{-1}$) | 122 ± 20.9 | 92 | 172 | 109.4 ± 36.5 | 43 | 216 | ns |
| Insulin (µUI $mL^{-1}$) | 10.4 ± 5.0 | 2.3 | 24.1 | 10.2 ± 3.6 | 3.8 | 14.7 | ns |

Note: The p-value was calculated using the chi-square test. For numerical variables, the mean is shown, and the t-test was used. n: number of subjects; SD: standard deviation; p-value ns: not significant. Abbreviations: DBP (diastolic blood pressure). SBP (systolic blood pressure). BMI (body mass index). gluc (glucose). AST (Aspartate aminotransferase). ALT (Alanine aminotransferase). GGT (γ-glutamyl transferase). TC (total cholesterol). TG (triglycerides). HDL-c (high-density lipoprotein cholesterol). LDL-c (low-density lipoprotein cholesterol).

A total of 43 volunteers 23 men and 20 women (aged between 31 and 63), regular red wine drinkers with an AUDIT score <8, and with at least one cardiovascular risk factor (high blood pressure, low HDL cholesterol, high fasting blood glucose, high triglycerides, increased waist circumference, or a BMI between 22.2 and 34.9 kg $m^{-2}$) completed the study (Table 2). A total of 22 subjects completed the intervention in the control wine group (9 women and 13 men), and 21 subjects completed the intervention in the GSPE wine group (11 women and 10 men). Baseline characteristics, after a washout period of 3 weeks without alcohol consumption, were comparable between groups, with no statistically significant differences observed for any evaluated parameter (Table 2).

To assess whether the volunteers experienced any significant dietary changes during the study, a questionnaire was administered that assessed the Mediterranean Diet Index for Chile at baseline and at the end of the study. The score obtained at the beginning of the study for the entire group was 6.4 points, and at the end of the study, 6.9 points, with no statistically significant change.

### 3.3 Compliance and tolerability

Among the 43 volunteers who completed the study, overall compliance with the recommended wine consumption was high. Strict adherence to the consumption protocol was reported by 65.1% of the participants (*n*= 28), while 16.3% reported missing wine consumption on up to 2 days (*n*= 7). Additionally, 18.6% of the participants (*n*= 8) reported consuming other alcoholic beverages on no more than 2 occasions during the intervention, primarily beer or sparkling wine, and always in moderate amounts. Compliance was assessed using daily participant logbooks, which were reviewed during follow-up assessments. Participants also received weekly telephone reminders to reinforce adherence.

Tolerability and overall satisfaction with wine consumption were generally favourable. Overall, 83% of participants rated their consumption experience as good or very good, mainly due to the pleasant taste, lower alcohol content, and their habitual wine consumption. A neutral evaluation was reported by 15% of participants, primarily because they were not accustomed to daily wine intake or missed consuming other alcoholic

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beverages. Only 2% of participants reported a poor experience, mainly due to dislike of the taste or an unpleasant mouthfeel. Reported adverse effects were mild and infrequent. Abdominal bloating and heartburn were reported by two participants consuming the GSPE wine (9.5%) and two consuming the control wine (9.0%). Headache was reported by two participants, although only one attributed it to wine consumption (4.5%). Dark stools were reported by two participants during consumption of the GSPE wine (9.5%).

**3.4 Effects of control and GSPE wine intake on clinical and biochemical parameters**

**Table 3** Effect of the control wine and non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine intervention on blood pressure, heart rate, waist circumference, weight, and BMI in the 43 volunteers who completed the study.

| | Control group (*n* =22) | | | GSPE wine group (*n* =21) | | | |
|---|---|---|---|---|---|---|---|
| Variable | Baseline | Final | $p^a$ | Baseline | Final | $p^a$ | $p^b$ |
| SBP (mmHg) | 120.5 ± 2.6 | 117.2 ± 2.6 | 0.165 | 123.0 ± 2.6 | 120.6 ± 2.6 | 0.32 | 0.79 |
| DBP (mmHg) | 78.7 ± 1.9 | 79.0 ± 1.9 | 0.884 | 82.3 ± 1.9 | 80.2 ± 1.9 | 0.245 | 0.349 |
| Heart rate (beats $min^{-1}$) | 71.0 ± 2.4 | 70.8 ± 2.4 | 0.881 | 71.2 ± 2.5 | 70.8 ± 2.5 | 0.807 | 0.944 |
| Weight (kg) | 75.8 ± 2.5 | 76.2 ± 2.5 | 0.142 | 80.6 ± 2.5 | 81.0 ± 2.5 | 0.097 | 0.867 |
| BMI (kg $m^{-2}$) | 26.8 ± 0.6 | 26.9 ± 0.6 | 0.16 | 28.0 ± 0.7 | 28.1 ± 0.7 | 0.101 | 0.844 |
| Waist circumference (cm) | 90.7 ± 1.8 | 90.5 ± 1.8 | 0.403 | 93.7 ± 1.8 | 93.7 ± 1.8 | 0.886 | 0.629 |

Note: values are expressed as mean ± standard error (SE) and were adjusted for sex and age. Comparisons were performed using linear mixed-effects models. $p^a$ indicates the comparison between final and baseline values within each group, whereas $p^b$ denotes the comparison of changes from baseline to final between the GSPE wine and control groups. A p-value < 0.05 was considered statistically significant.

The effects of control wine consumption compared to GSPE wine consumption on anthropometric, clinical, and biochemical parameters are shown in Tables 3-5 (*n*= 43, all volunteers who completed the study).

**3.5 Anthropometric, clinical and lipid profiles.** Table 3 shows the results for systolic and diastolic blood pressure, heart rate, waist circumference, weight, and BMI in the control wine group and the GSPE wine group. No statistically significant differences were observed in any of these parameters within the group or between groups when comparing final versus initial time.

Table 4 depicts the lipid profile parameters (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, non-HDL cholesterol, and triglycerides) in the control and GSPE wine groups. A slight increase in HDL cholesterol was observed in the control wine group; however, it did not reach statistical significance. No significant changes in the HDL levels were observed in the GSPE wine group. Meanwhile, no changes were detected in either group in total cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride concentrations when comparing the end point versus the start point.

**Table 4** Effect of the intervention with control wine and non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine on lipid profile parameters (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, non-HDL cholesterol, and triglycerides) in 43 volunteers who completed the study.

| | Control group (*n*= 22) | | | GSPE wine group (*n*= 21) | | | |
|---|---|---|---|---|---|---|---|
| Variable | Basal | Final | $p^a$ | Basal | Final | $p^a$ | $p^b$ |
| TC (mg $dL^{-1}$) | 196.2 ± 6.3 | 204.3 ± 6.3 | 0.095 | 184.3 ± 6.4 | 181.5 ± 6.4 | 0.565 | 0.115 |
| HDL-c (mg $dL^{-1}$) | 51.1 ± 2.6 | 53.7 ± 2.6 | 0.052 | 54.6 ± 2.7 | 53.8 ± 2.7 | 0.562 | 0.077 |
| LDL-c (mg $dL^{-1}$) | 121.6 ± 5.9 | 125.4 ± 5.9 | 0.334 | 109.2 ±6.0 | 105.4 ± 6.0 | 0.346 | 0.179 |
| VLDL-c (mg $dL^{-1}$) | 23.5 ± 2.8 | 25.2 ± 2.8 | 0.442 | 20.5 ± 2.8 | 21.9 ± 2.8 | 0.523 | 0.935 |
| Non-HDL-c (mg $dL^{-1}$) | 145.1 ± 6.8 | 150.7 ± 6.8 | 0.204 | 129.7 ± 6.9 | 127.2 ± 6.9 | 0.578 | 0.200 |
| TG (mg $dL^{-1}$) | 117.8 ± 13.8 | 125.5 ± 13.8 | 0.474 | 102.5 ± 14.0 | 109.4 ± 14.0 | 0.534 | 0.955 |

Note: values are expressed as mean ± standard error (SE) and were adjusted for sex and age. Comparisons were performed using linear mixed-effects models. $p^a$ indicates the comparison between final and baseline values within each group, whereas $p^b$ denotes the comparison of changes from baseline to final between the GSPE wine and control groups. A p-value < 0.05 was considered statistically significant. Abbreviations: TC (total cholesterol). TG (triglycerides). HDL-c (high-density lipoprotein cholesterol). LDL-c (low-density lipoprotein cholesterol).

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**Table 5** Effect of the intervention with control and non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine on hepatic profile parameters (AST, ALT, AST/ALT ratio, GGT, alkaline phosphatase, total bilirubin, direct bilirubin, and prothrombin time) in the 43 volunteers who completed the study.

| | Control group (*n*= 22) | | | GSPE wine group (*n*= 21) | | | |
|---|---|---|---|---|---|---|---|
| Variable | Basal | Final | $p^a$ | Basal | Final | $p^a$ | $p^b$ |
| AST (U $L^{-1}$) | 23.9 ± 2.0 | 27.4 ± 2.0 | 0.155 | 26.3 ± 2.1 | 24.1 ± 2.1 | 0.385 | 0.109 |
| ALT (U $L^{-1}$) | 25.0 ± 2.2 | 26.6 ± 2.2 | 0.325 | 31.2 ± 2.2 | 27.4 ± 2.2 | 0.022 | 0.021 |
| AST/ALT | 1.0 ± 0.082 | 1.2 ± 0.082 | 0.188 | 1.0 ± 0.083 | 0.9 ± 0.083 | 0.953 | 0.334 |
| GGT (U $L^{-1}$) | 19.1 ± 2.1 | 21.2 ± 2.1 | 0.067 | 21.2 ± 2.1 | 20.9 ± 2.1 | 0.771 | 0.135 |
| Alkaline phosphatase (U $L^{-1}$) | 74.0 ± 4.5 | 73.9 ± 4.5 | 0.983 | 83.5 ± 4.6 | 86.8 ± 4.6 | 0.138 | 0.279 |
| Total bilirubin (mg $dL^{-1}$) | 0.6 ± 0.053 | 0.5 ± 0.053 | 0.235 | 0.5 ± 0.054 | 0.5 ± 0.054 | 0.738 | 0.551 |
| Direct bilirubin (mg $dL^{-1}$) | 0.2 ± 0.017 | 0.2 ± 0.017 | 0.573 | 0.2± 0.018 | 0.2 ± 0.018 | 0.934 | 0.737 |
| Albumin (g $dL^{-1}$) | 4.7 ±0.03 | 4.6 ±0.03 | 0.030 | 4.6 ± 0.04 | 4.5 ± 0.04 | 0.184 | 0.551 |
| Prothrombin time (%) | 97.3 ± 2.2 | 100.6 ± 2.2 | 0.043 | 101.0 ± 2.3 | 103.1 ± 2.3 | 0.205 | 0.593 |

Notes: values are expressed as mean ± standard error (SE) and were adjusted for sex and age. Comparisons were performed using linear mixed-effects models. $p^a$ indicates the comparison between final and baseline values within each group, whereas $p^b$ denotes the comparison of changes from baseline to final between the GSPE wine and control groups. A p-value < 0.05 was considered statistically significant. Abbreviations: AST (Aspartate aminotransferase). ALT (Alanine aminotransferase). GGT (γ-glutamyl transferase).

**3.6 Hepatic profile**. Table 5 shows the hepatic profile parameters (AST, ALT, AST/ALT ratio, GGT, alkaline phosphatase, total bilirubin, direct bilirubin, albumin, and prothrombin time) in the control and GSPE wine groups. Although all hepatic parameters remained within normal reference ranges throughout the study, some changes were observed as a consequence of the intervention.

In the GSPE wine group, a significant decrease in ALT levels was observed when comparing final with baseline values (Table 5). Moreover, the comparison of changes from ALT levels at the baseline with final levels between control and GPW wine groups revealed a statistically significant difference for this parameter (p = 0.021), indicating a differential response between the intervention groups (Table 5). In contrast, the control wine group showed a significant increase in prothrombin time, whereas no significant change was observed in the GSPE wine group.

In addition, the levels of albumin showed a significant decrease at the end of the intervention in the control group. This parameter did not show statistically significant changes at the end of the intervention with GSPE wine. No changes were observed for albumin levels when the effects of the control group were compared with GSPE wine (Table 5).

No statistically significant changes were observed for the remaining hepatic parameters, either within groups or between groups, when comparing final and baseline values.

Plasma vitamin C levels and total antioxidant capacity, assessed by the TAR index, increased significantly after the control wine intervention (p = 0.029 and p = 0.003, respectively), with no significant changes observed in the GSPE wine group. When comparing changes from baseline between groups, a significant difference was observed only for TAR (p = 0.014).

Markers of oxidative damage to proteins (AOPP) and lipids (oxLDL) did not change significantly from baseline in either group, and no differences were detected between groups (Table 6). Tryptophan levels decreased significantly in both groups, whereas N-formylkynurenine and kynurenine decreased significantly only in the GSPE wine group. However, no significant differences between groups were observed for these variables.

No significant changes were observed in parameters related to glucose metabolism, including fluorescent AGEs at two wavelengths (AGEs 1 and 2), blood glucose, insulin, and the HOMA index, in either intervention group (Table 6).

## 4 Discussion

Red wine has been consumed by humans for millennia[50] and is still one of the most controversial components of the Mediterranean diet.[51] On the one hand, excessive consumption of alcohol has been associated with numerous adverse health outcomes, including cancer[52], liver diseases[53], and gout[54, 55], among others. On the other hand, a moderate

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**Table 6**. Effect of the intervention with control and non-fermented grape pomace skin-polyphenol-enriched (GSPE) wine on plasma levels of antioxidants-related parameters (uric acid, vitamin C, and TAR) and oxidative stress-related markers (AOPP, LDLox, AGEs, Dityr and Kyn).

| | Control group (*n*= 22) | | | GSPE wine group (*n*= 21) | | | |
|---|---|---|---|---|---|---|---|
| Variable | Basal | Final | p[a] | Basal | Final | p[a] | p[b] |
| Uric acid (mg $dL^{-1}$) | 4.9 ±0.2 | 5.4 ± 0.2 | 0.003 | 4.7 ± 0.2 | 4.6 ± 0.2 | 0.309 | 0.005 |
| Vitamin C (µM) | 53.5 ± 3.3 | 60.3 ± 3.3 | 0.029 | 47.3 ± 3.3 | 52.1 ± 3.3 | 0.133 | 0.629 |
| TAR (µM Equiv Tlx) | 228.4 ± 7.5 | 251.5 ± 7.5 | 0.003 | 207.5 ± 7.6 | 204.1 ± 7.6 | 0.613 | 0.014 |
| AOPP (nmol $mg^{-1}$ protein) | 67.9 ± 5.1 | 71.1 ± 5.1 | 0.548 | 63.2 ± 5.1 | 65.1 ± 5.1 | 0.716 | 0.873 |
| LDLox (ng $mL^{-1}$) | 101.4 ± 20.5 | 96.9 ± 20.1 | 0.350 | 56.1 ± 20.1 | 53.8 ± 20.8 | 0.633 | 0.753 |
| Trp | 864.8 ± 8.9 | 830.3 ± 8.9 | 0.009 | 869.1 ± 9.1 | 841.2 ± 9.1 | 0.036 | 0.715 |
| AGEs 1 | 19.89 ± 1.3 | 18.31 ± 1.3 | 0.360 | 22.3 ± 1.3 | 19.01 ± 1.3 | 0.064 | 0.480 |
| DiTyr | 15.55 ± 0.9 | 14.32 ± 0.9 | 0.378 | 17.6 ± 1.0 | 14.93 ± 1.0 | 0.071 | 0.487 |
| Kyn | 11.40 ± 0.7 | 10.47 ± 0.7 | 0.328 | 12.5 ± 0.7 | 10.50 ± 0.7 | 0.044 | 0.431 |
| AGEs 2 | 9.19 ± 0.5 | 8.66 ± 0.5 | 0.392 | 9.5 ± 0.5 | 8.70 ± 0.5 | 0.220 | 0.776 |

Note: Values are expressed as mean ± standard error (SE) and were adjusted for sex and age. Comparisons were performed using linear mixed-effects models. p[a] indicates the comparison between final and baseline values within each group, whereas p[b] denotes the comparison of changes from baseline to final between the GSPE wine and control groups. A p-value < 0.05 was considered statistically significant. Abbreviations: AST (Aspartate aminotransferase). ALT (Alanine aminotransferase). GGT (γ-glutamyl transferase). Equiv Tlx: Trolox equivalents. AOPP : advanced oxidation protein products. AGEs: advanced glycation end products.

intake of red wine has been shown to possess positive effects associated with the development of cardiovascular disease[13, 56], type 2 diabetes[57], a decrease in cognitive function, and all cause of mortality.[58] The mechanisms that could explain, in part, these positive effects have been attributed to the well-documented health benefits of phenolic compounds.[56, 57] Additionally, it has been proposed that moderate wine intake should be consumed with meals to minimize the risks of alcohol-related harm.[59] Considering this background, we aimed to formulate a low-alcohol, low-calorie wine enriched with non-fermented grape pomace skin polyphenols, in comparison with a control wine (low in alcohol and containing traditional polyphenol levels), and to evaluate the effects of its moderate consumption with meals on markers of metabolic syndrome, antioxidant profile, and oxidative damage in men and women.

The amount of polyphenols in wine has been estimated to be around 0.05–0.4 mg $mL^{-1}$ in white wines and 0.9–1.4 mg $mL^{-1}$ in young red wines, depending on numerous factors, including grape variety and conditions, as well as enological procedures.[60] In this study, the analysis of total polyphenols in the GSPE wine showed a higher content (4.26 ± 0.21 mg $mL^{-1}$) compared with reported values, as well as the control (low alcohol and calories) or original Malbec wine. The main differences when comparing the phenolic composition of GSPE wine with control wine were observed in malvidin-3-*O*-(6-*p*-coumaroyl) glucoside, petunidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside. These results are similar to the phenolic composition of grape pomace from *Vitis vinifera* of the cultivar Malbec, which showed that malvidin-3-glucoside was the main anthocyanin, followed by malvidin-3-*O*-*p*-coumaroylglucoside and petunidin 3-*O*-glucoside.[61]

The effect of a moderate daily intake of control wine and GSPE wine was assessed during approximately 40 days in overweight subjects or subjects with at least one risk factor for metabolic syndrome. We followed established definitions of moderate alcohol consumption, which differ between women and men due to physiological differences in alcohol metabolism. However, threshold values for moderate intake vary across studies. For example, Li et al. defined moderate consumption as 5–15 g $day^{-1}$ for women and 5–30 g $day^{-1}$ for men[62], while other studies and consensus have pointed out similar ranges, generally considering <15 g $day^{-1}$ for women and <30 g $day^{-1}$ for men as moderate intake[63, 64]. According to the Dietary Guidelines for Americans (2020–2025), moderate alcohol consumption is defined as up to one standard drink per day for women and up to two drinks per day for men[65]. In the present study, the administered wine provided approximately 8.1 g $day^{-1}$ of ethanol for women (125 mL $day^{-1}$) and 16.2 g $day^{-1}$ for men (250 mL $day^{-1}$). These values fall well within the range commonly considered as moderate alcohol consumption. The results show that moderate daily consumption of control and GSPE wine for approximately 40 days was safe and well-tolerated. No serious clinical adverse effects were observed, and none of the mild or moderate adverse effects were related to wine consumption. This is especially relevant in the case of GSPE wine, as daily consumption of GSPE wine with a high polyphenol content (4.26 mg GAE $mL^{-1}$) provides a total intake of 532 mg GAE $d^{-1}$ in women (125 mL) and 1065 mg GAE $d^{-1}$ in men (250 mL), compared to the control wine, with 274 mg GAE

$d^{-1}$ and 548 mg GAE $d^{-1}$, respectively. Recently, it has been estimated that the mean daily intake energy-adjusted of polyphenols is ~0.914 mg $kcal^{-1}d^{-1}$ in healthy adults (*n*= 350), reinforcing the high content of polyphenols in the GSPE wine. [66] In addition, the total polyphenols amount consumed by the volunteers in this study was similar to the daily intake estimated in different countries, ranging from 313.2 to 1740 mg $d^{-1}$.[67] No adverse effects were detected in biochemical, lipid, hepatic, or hematological profiles (data not shown). This finding is relevant as high polyphenol intake, particularly from concentrated supplements or extracts, may cause gastrointestinal discomfort such as diarrhea, likely due to limited absorption, osmotic effects, and microbial fermentation, whereas polyphenols consumed at normal dietary levels are generally well tolerated, as is shown in the case of GSPE wine.

Significant effects were observed as a consequence of dietary intervention with control wine and GSPE wine in parameters mainly related to liver function, antioxidant activity, and oxidative stress markers. A slight increase in HDL cholesterol was observed at the end of the intervention in the control wine group, bordering on statistical significance (p = 0.052). Alcohol consumption (ethanolic fraction) has been reported to raise HDL cholesterol levels.[13, 68] However, no significant changes were observed in the GSPE wine group. Notably, at the end of the intervention, participants consuming GSPE wine showed a significant decrease in ALT levels. High levels of ALT in the blood have been related to liver damage[69], and it has been reported to be strongly associated with non-viral cirrhosis.[70] In agreement with the results of this study, it has been reported that some polyphenols can reduce blood ALT levels in humans[71] and murine models.[72]

In this study, a significant increase in prothrombin time (expressed as percentage) was observed in the control red wine group, although still within the normal range (70- 120%), while the GSPE wine group showed no significant changes. Prothrombin activity is commonly used as an indicator of hepatic synthetic function because the liver produces several coagulation factors, including Factor I (fibrinogen), II (prothrombin), V, VII, and X. Alterations in the synthesis of these factors may impair coagulation, and are typically reflected by prolongation of prothrombin time or increases in the International Normalized Ratio (INR). Previous studies have reported changes in coagulation parameters following moderate red wine consumption, including modest increases in prothrombin activity. [73,74] The increase in prothrombin activity observed in the control wine group may reflect a mild modulation of coagulation parameters associated with moderate wine consumption, while the absence of changes in the GSPE wine group could suggest a differential effect related to the polyphenolic composition of the wines. However, since all values remained within normal ranges, these findings are unlikely to be clinically relevant.

Serum albumin levels also remained within the physiological range in both groups throughout the intervention. However, a significant within-group decrease was observed in the control wine group (p = 0.030), whereas no significant changes were detected in the GSPE wine group. Serum albumin is a marker of hepatic synthetic function and may be influenced by alcohol metabolism as well as inflammatory status.[75, 76] The decrease observed in the control group may reflect subtle physiological responses associated with alcohol metabolism, although values remained within normal range. In contrast, the absence of changes in albumin levels in the GSPE wine group may indicate that the higher polyphenol content of the wine may help preserve hepatic synthetic capacity. Polyphenols have been shown to attenuate alcohol-induced oxidative stress and inflammation in the liver, both of which can impair hepatocyte protein synthesis.[77] The potential protective effect of polyphenol-enriched wine on serum albumin levels may be mediated through several mechanisms, such as reducing reactive oxygen species production and modulating inflammatory signalling pathways (e.g., NF-κB, Nrf2, among others). Polyphenols may help preserve hepatocellular function and maintain albumin synthesis.[77] In addition, polyphenols can influence alcohol metabolism by modulating phase I and phase II enzymes, potentially limiting alcohol-related hepatic stress.[78] Together, these effects may contribute to the stability of serum albumin observed in the GSPE wine group.

Deviations from the protocol were classified as minor when they were infrequent, non-consecutive, and represented a negligible proportion of the total intervention exposure. It has been demonstrated that red wine intake can increase postprandial plasma polyphenol content and antioxidant activity[79]. However, under fasting conditions (12 h fasting), changes in plasma parameters such as TAR and uric acid following daily intake of 240 mL of red wine have been observed only after several weeks of sustained consumption[4]. Therefore, it is unlikely that these minor protocol deviations substantially altered the cumulative biological effects associated with the intervention. Considering that the control wine in this study was also low in alcohol and calories, with no statistically significant differences compared with the GSPE wine, the polyphenolic content and phenolic composition represent the main differences between the GSPE and control wines. The control wine group presented some significant changes previously described with moderate red wine consumption: increased levels of vitamin C, TAR, uric acid, and prothrombin activity.[4, 73, 74] The significant increase in vitamin C and TAR could be related to the increment in uric acid, since this purine metabolite product possesses antioxidant activity, which is consistent with previous studies.[80] The lack of significant effects observed in the antioxidant activity in the GSPE wine group could be partially explained by possible interaction between polyphenols and different organs.[81] The fact that plasma levels of Kyn, an oxidative stress marker, were significantly decreased in GSPE wine could suggest that this group was exposed to a lower oxidative stress or inflammation, since free Kyn is also a catabolite from Trp metabolism associated with inflammation[82], which should be analysed in future studies.

A limitation of this study was the sample size, which may restrict the generalizability of the findings to the wider population and their applicability to long-term outcomes. Another limitation of this study is the lack of assessment of polyphenolic bioavailability and metabolism, which is inherent to

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interindividual variability due to different microbiota composition, among other factors[32], that could influence the outcomes of the present study.

Finally, adherence to the dietary intervention represents a potential limitation of the study. As this trial was conducted under free-living conditions, perfect compliance could not be ensured despite the implementation of multiple monitoring strategies. Nevertheless, reported deviations of the protocol were minor and infrequent. Despite these limitations, the randomized, double-blind design and the use of a reduced-alcohol with normal polyphenol content control wine strengthen the robustness of the findings concerning intake of GSPE and the effects of polyphenols in the attenuation of alcohol-associated alterations related to hepatic function.

## 5 Conclusions

This study demonstrates that red wine reduced in alcohol and enriched with non-fermented grape pomace skin polyphenols was successfully formulated, preserving a high total polyphenolic content and antioxidant capacity. The intervention with GSPE wine was well tolerated and, compared with the control wine-reduced in alcohol, led to a favourable modulation of antioxidant-related and metabolic biomarkers in overweight subjects or participants presenting at least one component of the metabolic syndrome. These effects suggest that the enrichment with grape pomace polyphenols may enhance the functional properties of red wine beyond those attributable to alcohol reduction alone, particularly related to hepatic function. Further long-term and larger-scale studies are needed to confirm these effects and to elucidate the underlying mechanisms involved. These findings support the concept that technological strategies aimed at increasing the polyphenolic fraction of red wine while reducing alcohol content may represent a promising approach to improve the health-related functional properties of wine.

## Author contributions

Ines Urquiaga: methodology, investigation, writing – original draft, writing – review & editing, and project administration; Gerard Casaubon: conceptualization, resources, and funding acquisition; Druso Pérez: methodology and investigation; Daniela Sara: methodology and investigation; Pierre-Louis Teissedre: scientific advice and review & editing; Felipe Ávila: software, formal analysis, investigation, writing – original draft, and writing – review & editing. All authors have read and agreed to the published version of the manuscript.

## Conflicts of interest

G.C. is an employee of the Center for Research and Innovation, Viña Concha y Toro. A patent application related to the grape skin polyphenol extract used in this study has been filed with the Chilean National Institute of Industrial Property (INAPI) on 12 February 2026 (Application No. 2026004291).

## Data availability

Supplementary information is available.

## Acknowledgements

This work was funded by the project CORFO 22CVC2-218276 and Viña Concha y Toro.

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## Data Availability Statement

The anonymized raw data supporting the findings of this article are available from the corresponding author on reasonable request. The supporting data has been provided as part of the Supplementary information. Supplementary information: Tables S1 and S2, see DOI: [URL – format https://doi.org/DOI].