LWT - Food Science and Technology 258 (2026) 119962

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Dephenolisation and bleaching of defatted grape seed meal peptide hydrolysates: impact on chemical composition and antioxidant capacity

María del Rosario Rodríguez-Muñoz [a], Fabio Chinnici [b], Francisco J. Heredia [a], M. Lourdes González-Miret [a], María Jesús Cejudo-Bastante [a,*]

[a] Food Colour and Quality Lab., Facultad de Farmacia, Universidad de Sevilla, Sevilla, 41012, Spain
[b] Department of Agricultural and Food Sciences, University of Bologna, Bologna, 40126, Italy

ARTICLE INFO

Keywords:
Grape seed peptide hydrolysates
Dephenolisation
Bleaching agents
Wine colour stabilisation
Antioxidant capacity

ABSTRACT

Defatted grape seed meal (DGSM) can be hydrolysed to produce antioxidant soluble peptide hydrolysates to be used as colour stabiliser in red wines. This study focuses on the bleaching of hydrolysates to minimise the influence of its colour when added to wine. DGSM was dephenolised using 75% methanol (MeOH), 1% potassium metabisulphite (PMB) or sequential 75% MeOH+1% PMB. The most dephenolised sample was selected for further bleaching trials, using different agents and concentrations: polyvinylpolypyrrolidone (30 and 150 g/hL PVPP), activated charcoal (1 and 5 g/L AC), resin (25% and 50% *v/v* R), and a sequential treatment with 5 g/L AC followed by 50% R (AC + R). Dephenolisation process did not significantly ($p < 0.05$) affect the nitrogen content peptide. The highest dephenolisation and the lowest colour intensity was achieved with the sequential treatment. After bleaching, resin treatments resulted in the lowest content in nitrogen peptide (≈12–21% peptide loss) and amino acids, while R50 and AC + R produced the highest lightness and the lowest chroma. PVPP and low dose AC did not significantly reduce antioxidant activity. Overall, bleaching with AC5, R50, and AC + R produced promising bleached soluble peptide hydrolysates, with notable antioxidant capacity, producing viable candidates for further colour stabilisation in red wine.

1. Introduction

The food industry annually generates a significant volume of waste along the supply chain. In the EU alone, 55 million tonnes of food waste were produced in 2022, 20% of which came from processing and manufacturing, surpassed only by household waste (Eurostat, n.d.). An inadequate management of this waste contributes to greenhouse gas emissions and supports the lasted of pests and pathogens (Gómez-García et al., 2021). The EU has proposed legally binding goals to reduce food waste by 10% in processing and manufacturing by 2030 (European Commission, 2023). Therefore, the food industry is paying more attention on transforming residual waste into value-added products, encouraging the development of a circular economy that promotes resource efficiency and waste reduction.

Industries related to fruits and vegetables are responsible for considerable amounts of total food waste (Simões et al., 2021, pp. 619–644). The wine industry generates waste equivalent to approximately 25% of the total grape weight during vinification (Dwyer et al., 2014). Grape pomace is one of the most extensively explored by-products, accounting for 62% of total waste (Ruggieri et al., 2009), being a rich source of bioactive compounds, including lipids, phenolics, proteins, minerals, and dietary fibres (Chakka & Babu, 2022), with significant potential in food, cosmetic and pharmaceutical applications. Within grape pomace, the grape seeds are separated for oil extraction in the industry; however, the resulting defatted seeds, still contains significant protein fractions with potential for valorisation. These proteins have relevance to the food industry for their functional properties but also have technological attributes suitable for application in the industry (Cejudo-Bastante et al., 2022; De Angelis et al., 2024; Mora-Garrido et al., 2022; Rodríguez-Muñoz et al., 2025).

Climate change is having an impact on grape harvests in warm-climate regions, leading to a reduction of the phenolic compounds content, particularly anthocyanins and copigments, which play a crucial role in wine colour stabilisation through copigmentation (Mira de

* Corresponding author. Food Colour & Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012, Sevilla, Spain.
E-mail addresses: mrodriguez48@us.es (M.R. Rodríguez-Muñoz), fabio.chinnici@unibo.it (F. Chinnici), heredia@us.es (F.J. Heredia), miret@us.es (M.L. González-Miret), mjcejudo@us.es (M.J. Cejudo-Bastante).

https://doi.org/10.1016/j.lwt.2026.119962
Received 24 March 2026; Received in revised form 30 August 2026; Accepted 11 September 2026
Available online 12 September 2026

Orduña, 2010). This decrease makes it difficult to retain colour during storage and increases the need for strategies to enhance anthocyanin stabilisation. In this context, recent studies have scrutinized the positive effect of adding protein hydrolysates from defatted grape seed meals to wine during its stabilisation phase (Mora-Garrido, Escudero-Gilete, González-Miret, et al., 2024). The peptides present in the protein hydrolysate may interact non-covalently with phenolic compounds (López-Molina et al., 2025), enhancing copigmentation equilibrium and, subsequently, potentially contributing to colour stability.

The protein hydrolysate from defatted grape seed meals exhibits a characteristic brown colour (Mora-Garrido, Escudero-Gilete, Heredia, & Cejudo-Bastante, 2024; Rodríguez-Muñoz et al., 2025), similar to other protein hydrolysates derived from oilseeds, such as pumpkin or sesame seeds (Singh et al., 2022). This is particularly relevant since colour is one of the most important attributes influencing consumer perception and product acceptance. One reason for the brown colour may be the presence of phenols and their interaction with proteins (Tilley et al., 2023). The phenols present in the seeds oxidise and turn into quinones (electrophilic species) that, in presence of proteins (which have a nucleophilic amino group), form a brown quinone-protein adduct (Schieber, 2018). The brownish tonality could also be due to the formation of melanoidins (Sakai et al., 2022) produced by Maillard reaction, in which a reducing sugar reacts with an amino group of a protein and, through a series of subsequent steps, leads to the formation of brown polymers known as melanoidins (El Hosry et al., 2025). In this line, exploring decolouration strategies (through dephenolisation and bleaching) could be beneficial to improve the visual impact of protein hydrolysates and their suitability to be used in food applications.

These brown compounds, mainly associated with quinone-protein adducts and polymerised pigments, can be reduced or removed using a variety of different methodologies. Some methods are specifically designed to remove phenolic compounds, such as the extraction using 70% ethanol, which has been reported to achieve up to a 53% reduction in a canola seed protein (Jiang et al., 2021). Adsorption methods can also remove phenolic compounds, employing materials such as activated charcoal (Wang et al., 2025) or macroporous resins (Firdaous et al., 2017) to bind and remove these compounds from the hydrolysate matrix. Alternatively, oxidative bleaching, such as treatment with hydrogen peroxide followed by catalase, has been shown to be an effective method to reduce pigments derived from both phenolic compounds and Maillard reactions, without leaving chemical residues (Sakai et al., 2022). In practice, the challenge resides in achieving the most significant reduction in brown tonality without compromising the functional properties of the protein hydrolysate.

This work focused on achieving decolourisation of soluble peptide hydrolysates without compromising the protein content, involving a prior dephenolisation of defatted grape seed meal followed by a bleaching step of the soluble peptide hydrolysates. The main objective was to compare the effect of different bleaching agents at varying concentrations, in terms of their ability to reduce brown coloration and their influence on the chemical characteristics (the colorimetric characteristics, the nitrogen peptide content, amino acid content, antioxidant capacity, and peptide profile) of the bleached peptide hydrolysates. This study could represent an important advance in warm-climate oenology, since permits to establish an optimal procedure for obtaining peptide hydrolysates focused on the red wine color stabilisation.

2. Materials and methods

2.1. Chemical and reagents

The endoprotease Novo-ProD® was supplied by Novozymes (Copenhagen, Denmark). The bleaching agents were provided by Agrovin S.A. (Alcázar de San Juan, Ciudad Real, Spain): activated charcoal as a black powder with 75–100% carbon purity, cation exchange resin as a brown-black monodisperse bead gel with a total exchange capacity (Na^+ form) of 2.05 eq/L, and PVPP as a white powder with 75–100% polyvinylpyrrolidone purity). 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were provided by Sigma–Aldrich (Madrid, Spain), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Alfa Aesar (Ward Hill, MA, USA). Methanol and potassium metabisulphite were supplied by Honeywell (Charlotte, NC, USA) and Enartis (San Martino di Trecate, Novara, Italia), respectively. 30% ammonia and 96% sulphuric acid were acquired by Panreac (Barcelona, Spain), and sodium hydroxide and hydrochloric acid were purchased from Fluorochem (Derbyshire, UK), and VWR (Radnor, PA, USA), respectively.

2.2. Samples

The starting material, defatted grape seed meal (DGSM) from the varieties Tempranillo (red grape) and Airén (white grape), was supplied by Alvinesa Natural Ingredients S.A (Daimiel, Ciudad Real, Spain). DGSM is an already defatted residue (0.4% fat) generated during the industrial processing of grape pomace, obtained after extracting components of interest as alcohols, phenols, and bitartrate. After washing and drying, grape seeds are separated, grounded, and subjected to oil extraction using hexane, which were further desolventised to obtain DGSM (Mora-Garrido et al., 2022). The DGSM was ground in the laboratory with a mill (IKA A11-B, IKA®, Staufen, Germany), to facilitate the protein extraction, and stored in darkness at room temperature. DGSM used in all assays came from the same industrial batch.

2.3. Dephenolisation of DGSM

Some phenols are coloured molecules and may influence the colour of the sample. On the other hand, phenols can act as copigments in stabilising the colour of red wine, so their presence could interfere with the interpretation of the interactions between the peptide and the anthocyanin pigments in red wine. Therefore, it is important to remove phenolics from DGSM before to proceed to their bleaching.

Following the adapted method of Jara-Palacios et al. (2014), two solvents were tested, individually and sequentially applied, to evaluate their efficacy. Three extraction assays (each in triplicate) were performed on DGSM: i) 75% (*v/v*) methanol, ii) 1% (*w/v*) potassium metabisulphite, and iii) a sequential extraction with 75% methanol followed by 1% potassium metabisulphite. 4 g of DGSM was mixed with 10 mL of extraction solvent and shaken at 2500 rpm on a VXM-TDGB multi-tube vortex mixer (Ohaus, Nänikon, Switzerland). Then, samples were centrifuged at 4000 rpm for 5 min at 4 °C (Microfuge® 22R, Beckman Coulter™, Brea, CA, USA) to separate the solid residue (dephenolised DGSM) from the supernatant (phenolic extract). The procedure was subsequently repeated twice using 5 mL of the extraction solvent. In the sequential assay (iii), 20 mL of 1% potassium metabisulphite was applied to the solid residue previously dephenolised with 75% methanol, following the same procedure described above. Subsequently, the pooled supernatants for each treatment were adjusted to a final volume of 25 mL with their respective extraction solvents. Thus, the extraction assays resulted in three phenolic extracts, those obtained with 75% methanol, with 1% potassium metabisulphite, and with the sequential procedure, and the corresponding three dephenolised meals (DM), by using 75% methanol (DMM), 1% PMB (DMS), and the sequential extraction (DMMS). A non-dephenolised DGSM was considered as control.

2.4. Obtaining soluble peptide hydrolysate from DGSM

Soluble peptide hydrolysate was obtained as described by Rodríguez-Muñoz et al. (2025). The DGSM used for hydrolysis was previously dephenolised with 75% of methanol followed by 1% potassium metabisulphite. Seven hundred g of dephenolised DGSM, was

mixed with 3.5 L of distilled water (20:80, w/v) in a 7 L bioreactor (Bio Console ADI 1025, Applikon Biotechnology, Inc., Delf, Netherlands) and was kept in constant agitation on basic medium (3 h, 180 rpm, 25 °C, pH 10.5 using 30% NH_3). The non-protein fraction (precipitate) was separated and discarded by centrifugation (20 min, 14,880 × *g*, 4 °C) (Avanti J-26 XP Centrifuge, Beckman Coulter, California, USA), and the soluble proteins (supernatant) was concentrated under vacuum in a rotary evaporator (60 °C, 1 h) to eliminate NH_3, and subsequently decreasing pH, and thus prevent the further formation of salts. The resulting protein concentrate was heated in a water bath to inactivate endogenous enzymes (80 °C, 5 min) and then submitted to enzymatic hydrolysis in a 3 L bioreactor using 0.6 *v/v* Novo-ProD® endoprotease enzyme under optimal conditions (pH 9, 60 °C, 2 h) (Rdsa et al., 2018). The crude hydrolysate obtained was concentrated in a rotary evaporator at 80 °C until the volume was reduced by about half. The pH was adjusted to 3.5 (usual pH of wine) using 32% HCl to induce the precipitation of non-hydrolysed peptides. The supernatant (soluble peptides hydrolysate) was separated by centrifugation (15,000 × *g*, 4 °C, 20 min), and the precipitate was discarded. Approximately 0.5 L of soluble peptide hydrolysate was obtained, filtered through filter paper to remove possible residues, and used for all subsequent analyses.

2.5. Bleaching process of peptide hydrolysates

Three bleaching agents at two different concentrations (following the manufacturer's recommendations), were applied: polyvinylpolypyrrolidone (PVPP, 30 and 150 g/hL), activated charcoal (AC, 1 and 5 g/L), and resin (R, 25% and 50% (*v/v*); a sequential treatment (5 g/L AC followed by 50% R) was also evaluated. The bleaching procedures were performed, in triplicate. The bleaching agents was added to 100 mL of dephenolised soluble peptide hydrolysate, previously obtained, and stirred (800 rpm) on a magnetic stirrer (Nahita-Blue 692; Auxilab S.L., Navarra, Spain). In the case of activated charcoal, the mixture was centrifuged (3000 × *g*, 5 min) to separate the particles of charcoal from the supernatant. All solutions were filtered through 0.45 μm mixed cellulose ester filter (MF-Millipore™, Merck, Darmstadt, Germany). The resulting solutions were then concentrated under vacuum in a rotary evaporator (60 °C) and lyophilised for further characterisation. Thus, seven DEd soluble peptide hydrolysates resulted: obtained with PVPP (PH-PVPP30 and PH-PVPP150), activated charcoal (PH-AC1 and PH-AC5), resin (PH-R25 and PH-R50), and charcoal + resin (PH-AC5+R50). A non-bleached soluble peptide hydrolysate was considered as control (PH-C).

2.6. HPLC-DAD analysis of polyphenolic compounds of DGSM

1 mL of phenolic extract was filtered by 0.45 μm nylon filter prior to injection for chromatographic analysis. The identification and quantification of phenolics (flavan-3-ols, and hydroxycinnamic acid, benzoic acid and flavonol derivatives) was performed according to Jara-Palacios et al. (2020). Analyses were carried out using an Agilent 1290 chromatographic system, equipped with a quaternary pump, a UV–vis diode array detector (scan 200 to 770 nm), an automatic injector and the ChemStation software (Palo Alto, CA, USA). 1.5 μL was injected onto a C18 Eclipse Plus 120 column (1.8 μm, 50 × 2.1 mm), maintained at 25 °C. The solvents were 0.01% formic acid (eluent A) and acetonitrile (eluent B), the flow rate was 1.0 mL/min. The elution gradient was: 0–5 min, 5% B; 5–20 min, 50% B; and 20–25 min, 100% A. Detection and quantification wavelengths were set at 280 nm for flavan-3-ols and benzoic acid derivatives, 320 nm for hydroxycinnamic acid derivatives, and 360 nm for flavonol derivatives, by comparing the areas and retention times with the standards of (+)−catechin, gallic acid and protocatechuic acid, caffeic acid and quercetin, respectively, using calibration curves. Quantification was carried out in triplicate, and the results were expressed as total concentration of each phenolic family computed as the sum of the content of individual compounds (μg/g DGSM).

2.7. Colorimetric analysis

Colour was determined by diffuse reflectance using a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan), following the recommendations of the Commission Internationale de l'Eclairage (CIE, 2004). CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, and h_{ab}) were calculated considering the CIE 1964 10° Standard Observer and Standard Illuminant D65 as references. Measurements were performed in triplicate. Moreover, colour differences (ΔE^*_{ab}) between pairs of samples were calculated as the Euclidean distance between two points in the three-dimensional CIELAB colour space, according to the CIE 1976 colour difference formula:

$$\Delta E^*_{ab} = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* correspond to the differences in lightness (L^*), a^* and b^* between pairs of samples.

The relative contributions of lightness (% $\Delta^2 L$), chroma (% $\Delta^2 C$), and hue (% $\Delta^2 H$) to the colour difference were calculated as quadratic differences and expressed as percentages:

$$\%\ \Delta^2 L = \left[(\Delta L^*)^2 \,/\, (\Delta E^*_{ab})^2\right] \times 100 \quad (2)$$

$$\%\ \Delta^2 C = \left[(\Delta C^*_{ab})^2 \,/\, (\Delta E^*_{ab})^2\right] \times 100 \quad (3)$$

$$\%\ \Delta^2 H = \left[(\Delta H)^2 \,/\, (\Delta E^*_{ab})^2\right] \times 100 \quad (4)$$

ΔH is deduced mathematically from $\Delta H = \sqrt{\left(\Delta E^*_{ab}\right)^2 - (\Delta L^*)^2 - \left(\Delta C^*_{ab}\right)^2}$ (5)

2.8. Nitrogen content of peptide (%)

The total nitrogen content was determined by the standard Kjeldahl method (AOAC, 1995) using an Automatic Kjeldahl Distiller system (J.P. Selecta, Barcelona, Spain). The nitrogen content of peptide (%) was calculated based on the nitrogen content, considering a conversion factor of 5.75 (Cejudo-Bastante et al., 2022). The peptide yield was also calculated (nitrogen content of peptide (%) × sample weight (g))/(DGSM weight (g)). All determinations were performed in triplicate.

2.9. Ion-exchange chromatography analysis of amino acid content

The amino acid profile (individual and total content) was determined by ion-exchange chromatography with post-column derivatisation using ninhydrin (Cejudo-Bastante et al., 2022). 5 mg of each sample were hydrolysed in 1 mL of 6 M HCl at 110 °C for 24 h. After hydrolysis, the samples were centrifuged (11,357 × *g*, 4 °C, 15 min), and the acidic supernatants (pH ≤ 1) were adjusted to pH 2.0 with 6 M and 1 M NaOH. Afterwards, 400 μL was mixed with 100 μL of 50 μM norleucine (internal standard) and filtered through 0.45 μm nylon filters. Finally, the analysis was carried out on a Biochrom 30+ Amino Acid Analyser (Biochrom Ltd., Cambridge, UK) equipped with a high-pressure PEEK cation-exchange column packed with Ultropac 8 resin and a UV–vis detector. The wavelengths for detection were 440 nm for proline and 570 nm for all other amino acids. Quantification of amino acids was performed in triplicate, and results were expressed as mg/g of soluble peptide hydrolysate.

2.10. Peptide composition

Peptides present in the studied soluble peptide hydrolysates were qualitative identified by RP-LC-MS/MS. The sample (6 mg) was

dissolved in 1 mL of ultrapure water (Milli-Q), filtered through a nylon filter (0.45 μm) and analysed in an EASY-nLC II system attached to an ion trap LTQ-Orbitrap-Velos-Pro hybrid mass spectrometer (Thermo Scientific). Then, the peptides were concentrated by reverse phase chromatography using a C18 RP precolumn (20 mm × 100 μm inner diameter, 5 μm particle size, 100 Å pore size; Thermo Scientific), and then separated using a C18 RP column (250 mm × 75 μm inner diameter, 3 μm particle size, 100 Å pore size; Thermo, Scientific) at a flow of 0.3 μL/min. Subsequently, a 180-min chromatographic run was applied using the following gradient: 5–25% solvent B over 135 min, 25–40% solvent B over 45 min, 40–100% solvent B over 2 min, followed by 100% solvent B for 18 min (solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid, 80% acetonitrile in water) (Cejudo-Bastante et al., 2022). Electrospray ionization was conducted employing a Nano-Bore Emitters Stainless Steel (30 μm ID; Proxeon) at 2.1 kV with a 60% S-Lens. Full scan MS spectra were acquired at a resolution of 30,000 over the *m/z* range of 400–1600 (1 μscan), followed by 20 data dependent MS/MS events (Top 20). The MS/MS isolation width was 2 units (in mass-to-charge ratio units), with a normalised collision energy of 35%, and dynamic exclusion was pursued for periods of 60 s. Charge-state screening was enabled to discard unassigned and singly charged protonated ions. The results were matched with the UniProt database and Peaks X Pro software (version X Pro). The analyses were performed in triplicate.

2.11. Antioxidant activity

Three different methods were performed to evaluate the antioxidant capacity. DPPH and ABTS are spectrophotometric methods based on the capacity to scavenge radicals through hydrogen atom or electron transfer (Baliyan et al., 2022; Bessada et al., 2015; Hernández-Rodríguez et al., 2019, pp. 265–288). The absorbances were measured in an Agilent 8453 UV-vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). BRS system is an electrochemical technique based on the voltametric charge of the sample after electrochemical oxidation.

2.11.1. DPPH assay

The DPPH assay was conducted according to Soler-Rivas et al. (2000). Briefly, 0.02 g of sample was dissolved in 10 mL of PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 137 mM NaCl, pH = 7.4). To prepare the Trolox calibration curve, 30 μL of standard Trolox was mixed with 300 μL of 108 μM DPPH methanolic solution, and 570 μL of 80% (*v/v*) methanol. The reagent blank and samples were prepared under the same conditions using 80% (*v/v)* of methanol or sample solution, respectively. Subsequently, a pre-dilution test was performed using 1/1 and 1/2 dilutions of samples, to ensure that absorbance was within the calibration curve range. After 30 min incubation (room temperature at dark), absorbances were measured at 515 nm. The results were expressed as Trolox equivalents (TE)/g of soluble peptide hydrolysate. All determinations were made in triplicate.

2.11.2. ABTS free radical scavenging assay

The ABTS method was performed according to Re et al. (1999), with some modifications. The $ABTS^{\bullet+}$ stock solution (7 mM) was prepared with 2.45 mM potassium persulfate as oxidising agent, keeping it in the dark at room temperature for 12–16 h. The stock solution was then diluted with PBS (1 mL of ABTS and 79 mL of PBS) to obtain the working solution, adjusting the absorbance to 0.700 ± 0.020 at 734 nm. PBS was considered as blank. Subsequently, a pre-dilution test was performed on the samples, using dilutions of 1/1 and 1/2 in triplicate. Finally, 50 μL of sample was mixed with 2 mL of the working reagent, measuring the absorbance at 734 nm. The results were expressed as Trolox equivalents (TE)/g of soluble peptide hydrolysate. The measurements were carried out in triplicate.

2.11.3. Electrochemical antioxidant capacity

The antioxidant activity was also performed, in triplicate, using a BRS device (Bioquochem Redox System) supplied by BQC Redox Technologies (Oviedo, Spain), an electrochemical system working with disposable screen-printed electrodes. A previous test was conducted to determine the optimal working concentration and solvent for the analysis. Based on the BRS values reported by Corpas et al. (2024) for red fruits, 6 mg of samples was dissolved in 1.5 mL of the provided electrolyte solution. 100 μL of this mixture was placed on the electrode for measurement. The results were expressed as Trolox equivalents (TE)/g of soluble peptide hydrolysates. The antioxidant activity was determined in triplicate.

2.12. Statistical analysis

Statistical analysis was performed using Statistica v.8.0 software (StatSoftInc., 2007). To ensure the validity of statistics applied to the results, normality was evaluated using Shapiro Wilk test, and homoscedasticity of variances using Levene's test. Subsequently, a one-way analysis of variance (ANOVA) was applied to establish statistical differences among treatments and samples, followed by a Tukey post hoc test. Principal component analysis (PCA) was also conducted, using the correlation matrix to standardise variables, to identify the main contributors to variance. Furthermore, a Pearson's correlation analysis was applied to evaluate if there is a relation between the parameters.

Throughout the text, the term 'significant' is used only to indicate statistical significance (p-values < 0.05).

3. Results and discussion

3.1. Dephenolisation of DGSMs

3.1.1. Determination of phenolic compounds of DGSM

The phenolic content of the extracts obtained from DGSM were determined, including total and individual compounds belonging to four phenolic families: flavan-3-ols (catechin derivatives), hydroxycinnamic acid derivatives (HCAD) (coumaric acid derivatives and other derivatives), benzoic acids (gallic acid and protocatechuic acid, and their derivatives), and flavonols. In general, the phenolic content was low (0.1-0.5 μg/g) (Fig. 1), substantially lower than that typically found in grape seeds (Mora-Garrido et al., 2022), since DGSM has already undergone exhaustive extraction of compounds (phenols, among them) by the grape pomace industry. Regardless of the extraction solvent, the benzoic acids family showed the highest concentration, followed by flavan-3-ols and HCAD.

The extracts obtained with 75% MeOH contained the highest concentration of total phenols (0.50 μg/g), showing significant differences compared with those obtained with 1% PMB (0.28 μg/g) (Fig. 1), which is in accordance with Jara-Palacios et al. (2014). As expected, the second extract of the sequential treatment conducted to extracts with the significantly lowest content (0.1 μg/g).

Considering individual compounds, the extracts obtained with 75% MeOH showed significantly higher concentration of flavan-3-ols, HCAD, and benzoic acids, mainly due to the presence of gallic acid and protocatechuic acid derivatives (0.14 μg/g in both cases). These results highlight the strong extraction capacity of this solvent. In contrast, the extracts obtained with 1% PMB not only contained lower content of benzoic acids, but also exhibit different extraction profile, recovering different benzoic acid compounds compared with 75% MeOH, specifically gallic and protocatechuic acids (0.13 and 0.09 μg/g, respectively). These findings indicate that PMB displayed a certain selectivity for these phenolics (Jara-Palacios et al., 2020), which was further confirmed when the DGSM dephenolised with 75% MeOH was submitted to additional dephenolisation with 1% PMB, and only gallic and protocatechuic acids were found in the extract.

Therefore, the sequential application of 75% MeOH followed by 1%

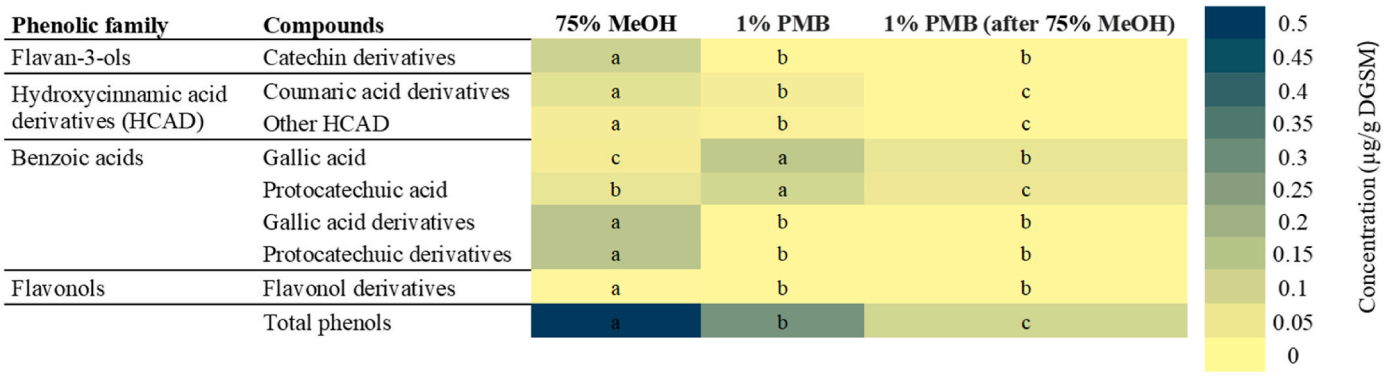

Fig. 1. Heat map of the content of phenolic compounds of DGSM extracts, obtained with different solvents 75% MeOH, 1% PMB, and 1% PMB after 75% MeOH. Coloured cells encode concentration (µg/g DGSM). For each phenolic compound, different letters indicate significant ($p < 0.05$) differences among solvents according to Tukey's test.

PMB resulted in a virtually complete extraction (recovery) of the phenolic content, proving to be the most effective process for dephenolisation, and ensuring the production of a dephenolised DGSM.

3.1.2. Colorimetric analysis of dephenolised DGSMs

Phenolic compounds participate in oxidation reactions eventually giving rise to brown pigments, which could contribute to the dark brown colour of DGSM (Yu et al., 2024). Thus, the removal of these compounds is expected to yield a DGSM with a lighter, less saturated colour with a reduced contribution of brown tonality. The colour of the dephenolised DGSMs (DMM, DMS, and DMMS) was measured to evaluate the impact of phenolic removal, compared to the control meal (DGSM, non-dephenolised). The CIELAB units ranged from 36.49 to 42.76 for lightness (L^*), 16.23 to 18.83 for chroma (C^*_{ab}), and 56.08 to 60.41 for hue (h_{ab}) (Table 1). Based on the CIELAB parameters (L^*, C^*_{ab}, and h_{ab}), DMM displayed the lowest lightness (L^*, 36.49) values, followed by DGSM (L^*, 38.96), producing the darkest defatted grape seed meals, probably due to the MeOH-induced darkening. In contrast, DGSM treated with PMB (DMS) resulted in the lightest dephenolised samples (L^*, 42.76), and the sequential use of MeOH and PMB (DMMS) produced intermediate lightness values (L^*, 40.51). Therefore, the effect of dephenolisation on the lightness of samples is highly dependent on the solvent used. Furthermore, the phenolic removal diminishes colour intensity, which is evidenced in a decrease of chroma in all the dephenolised samples. Among them, DMMS samples had the lowest colour intensity (C^*_{ab}, 16.23). The increased lightness and reduced colour intensity observed when PMB is used (DMS and DMMS) can be attributed to the significantly extraction of gallic and protocatechuic acids compared with the extraction with 75% methanol. A possible additional contribution is the reaction of sulphite with phenolic quinones (brown pigments), which leads to the formation of colourless adducts and a consequent reduction of browning (Narváez-Cuenca et al., 2011). Regarding hue (h_{ab}), although small differences exist among samples, those treated with 1% PMB (DMS and DMMS) displayed significantly higher values towards yellow hue (h_{ab}, 60.41 and 59.47, respectively), mainly in samples treated exclusively with this solvent. Overall, DMMS could be the best option for subsequent hydrolysate obtention, as it is expected to have the least contribution on the CIELAB parameters of the final product, in the light of its lighter and less yellow saturated colour.

The effect of dephenolisation treatment on colour was assessed by pairwise comparisons with DGSM using the colour difference formula (ΔE^*_{ab}) (Fig. 2). All dephenolisation methods produced colour differences (ΔE^*_{ab}) up to >3 CIELAB units, regardless of the solvent (Fig. 2). The colour differences produced by MeOH (DMM) affected predominantly to quantitative colour parameters, with lightness ($\%\Delta^2 L$) being the main contributor, followed by chroma ($\%\Delta^2 C$). The changes occurred by dephenolisation with 1% PMB are mainly due to the qualitative colour parameter, that is, the hue ($\%\Delta^2 H$) (55%), followed by lightness (42%). Furthermore, dephenolisation with 1% PMB after 75% MeOH treatment (DMMS) also generate colour differences, mainly

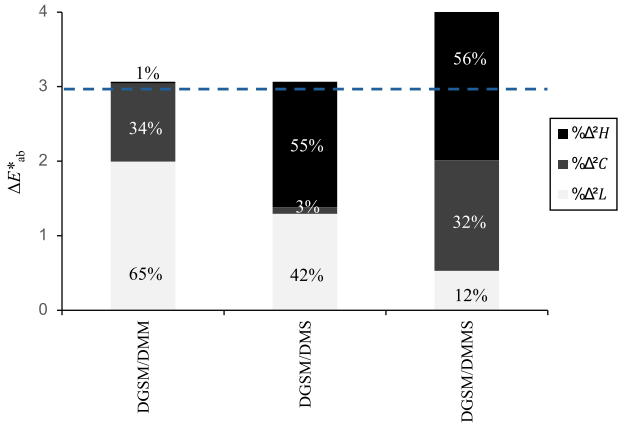

Fig. 2. Colour differences (ΔE^*_{ab}) and percentages of the relative contributions of lightness, chroma, and hue ($\%\Delta^2 L$, $\%\Delta^2 C$, and $\%\Delta^2 H$) of grape seed meal (DGSM) and dephenolised grape seed meal with 75% MeOH (DMM), 1% PMB (DMS), and 75% MeOH+1% PMB (DMMS).

Table 1
CIELAB colour parameters (L^*, C^*_{ab}, h_{ab}) and nitrogen content peptide (%) of control and dephenolised grape seed meal. Mean ± standard deviation (n = 4).

	Colour parameters (CIELAB units)											Nitrogen content peptide (%)			
	L^*				C^*_{ab}				h_{ab}						
DGSM	38.96	±	0.86	c	18.83	±	0.28	a	56.08	±	0.21	c	8.78	±	0.31	a
DMM	36.49	±	0.28	d	17.04	±	0.54	bc	56.36	±	0.33	c	8.19	±	0.45	a
DMS	42.76	±	0.25	a	17.72	±	0.11	ab	60.41	±	0.12	a	8.11	±	0.28	a
DMMS	40.51	±	0.60	b	16.23	±	0.50	c	59.47	±	0.28	b	8.06	±	0.22	a

DGSM, control non-dephenolised; Dephenolised grape seed meals: DMM (treated with 75% MeOH), DMS (treated with 1% PMB), DMMS (treated with 75% MeOH + 1% PMB). Different letters in the same column indicate significant ($p < 0.05$) differences among samples according to Tukey's test.

qualitative due to hue (56%), but also quantitative, due to chroma (32%), resulting in a paler yellowish appearance. These results suggest that the compounds extracted with the different solvents provide distinct contributions to the colour differences. Therefore, the sequential use of both solvents could be considered the most suitable option due to the higher lightness and lower chroma of dephenolised DGSM, achieving both qualitative and quantitative contributions to colour differences.

3.1.3. Nitrogen content peptide (%) of the dephenolised DGSMs

Table 1 shows the nitrogen peptide content of all dephenolised samples, being approximately 9%, which is consistent with Mora-Garrido et al. (2022) for similar raw material. Not-significant ($p > 0.05$) differences were observed among samples. These results indicate that dephenolisation treatment did not affect the nitrogen peptide content, regardless of the solvent used.

3.2. Bleached peptides hydrolysates

Based on the results of the dephenolisation assay, the DMMS (grape seed meal dephenolised by using sequential treatment with MeOH and PMB) was selected to perform the bleaching assay. The DMMS was hydrolysed, and the soluble peptide hydrolysate was submitted to different bleaching treatments using different concentrations of polyvinylpolypyrrolidone (PVPP), activated charcoal (AC) and resin (R). The characteristics of the resulting bleached products were determined.

3.2.1. Colour properties

All hydrolysates were in the first quadrant of the (a*b*)-plane (Fig. 3), corresponding to brownish hues, similarly to that reported by Mora-Garrido, Escudero-Gilete, Heredia, and Cejudo-Bastante (2024) and Rodríguez-Muñoz et al. (2025). The values of the CIELAB colour parameters (L^*, C^*_{ab} and h_{ab}) of unbleached and bleached peptides hydrolysates are shown in Table 2. Significant differences were observed for the hydrolysates bleached with AC and R at their highest concentrations, both individually and in combination (PH-AC5, PH-R50 and PH-AC5+R50). Small and not-significant colour changes occurred with the other treatments (PVPP, regardless of the concentration, and AC and R at low proportions).

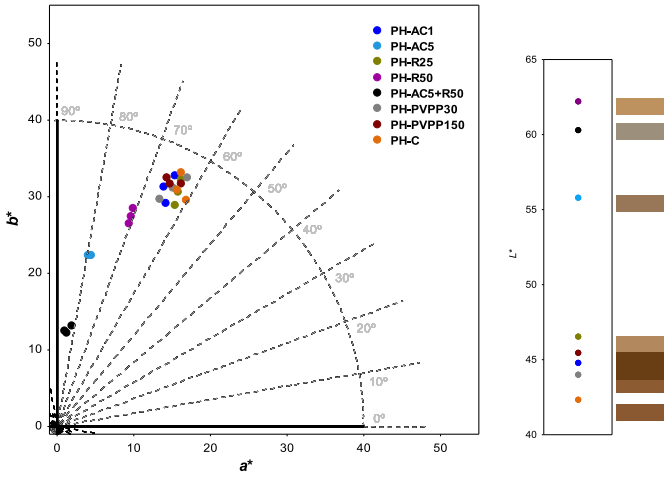

Fig. 3. Representation of samples in the (a^*b^*) plane and their lightness (L^*) values. PH-C, control unbleached soluble peptide hydrolysates; PH-PVPP30, soluble peptide hydrolysates treated with 30 g/hL PVPP; PH-PVPP150, peptide hydrolysates treated with 150 g/hL PVPP; PH-AC1, soluble peptide hydrolysates treated with 1 g/L activated charcoal; PH-C5, soluble peptide hydrolysates treated with 5 g/L activated charcoal; PH-R25, soluble peptide hydrolysates treated with 25% resin; PH-R50, soluble peptide hydrolysates treated with 50% resin; PH-C5+R50, soluble peptide hydrolysates treated with 5 g/L μmol Tx/g soluble peptide hydrolysate activated charcoal + 50% resin.

The lightness (L^*) values increased in all bleached hydrolysates, achieving the highest one with the sequential treatment (PH-AC5+R50). The chroma (C^*_{ab}) values showed a similar behaviour to that of L^*, in this case with a decrease in values compared to the control. The highest reduction of colour intensity occurred in the sequential treatment (64%), followed by AC5 (36%) and R50 (26%) bleaching treatments. Regarding hue values (h_{ab}), bleaching induced changes towards yellow tonalities ($h_{ab} \approx 90°$) with lower contribution of red colour ($h_{ab} \approx 0°$). Once again, the highest increase of hue was observed in PH-AC5+R50, resulting in the greatest reduction of the typical brown colour of these samples (Mora-Garrido, Escudero-Gilete, Heredia, & Cejudo-Bastante, 2024; Rodríguez-Muñoz et al., 2025), followed by PH-AC5 and PH-R50, being significant increases in the three cases. Consequently, due to their reduced colour, PH-AC5, PH-R50 and PH-AC5+R50 could serve as colour stabilisers to be used in winemaking, helping to preserve the original wine colour and prevent undesirable brownish tonalities over time.

3.2.2. Nitrogen content peptide (%)

The nitrogen content peptide (%) of the control (unbleached) and the bleached soluble peptide hydrolysates are summarised in Table 2. The protein content of PH-C (control) was 52%, which is similar to values reported for non-dephenolised protein hydrolysates from defatted grape seed meals (Mora-Garrido et al., 2022). Regarding the bleached hydrolysates, Table 2 shows that nitrogen peptide content was influenced by the type and concentration of bleaching agent. The treatments with 30 g/hL PVPP and AC at both concentrations did not affect the nitrogen content peptide (not-significant differences were found compared to control), which, in terms of preserving protein content, indicate adequate bleaching treatments of the hydrolysate for its subsequent use in winemaking. In contrast, the use of 150 g/L PVPP, 25% resin and 50% resin provoked significant nitrogen peptide reductions compared to control (around 5%, 18% and 12%, respectively). The higher reduction in protein content (21%) occurred in the sequential treatment (AC + R).

These results can be explained based on the different affinities of the agents used. As described in the literature, although ion-exchange resins establish π–π stacking interactions with phenols, the electrostatically affinity for charged proteins and peptides appears to be more pronounced (de Bruijn et al., 2009; Pismenskaya et al., 2020). Furthermore, activated charcoal affects both phenolic compounds (Villacañas et al., 2006) and large peptides and proteins (Xiao et al., 2014) by stablishing different types of interactions (π–π interactions, electron donor, hydrogen bonding or electrostatics, respectively) (Lorenc-Grabowska, 2015; Xiao et al., 2014). PVPP mainly shows affinity for polyphenols (Postai et al., 2025) through a combination of hydrogen and hydrophobic bonding (Siebert & Lynn, 2007).

A direct relationship was observed between the decrease in nitrogen content peptide and the colour changes. The treatments that led to the largest peptides lost, resin (PH-R25 and PH-R50) and the sequential use of both agents (PH-AC5+R50), also resulted in the highest lightness and lowest chroma values.

3.2.3. Amino acid composition

The amino acid composition of the soluble peptide hydrolysates (control and bleached) was determined, including individual and total amino acids (TAA) and their classification into sulfur (SAA: Cys and Met), hydrophobic (HAA: Ala, Val, Met, Ile, Leu, and Phe), and aromatic (AAA: Tyr, Phe, and His) amino acids (Table 3). It can be observed that Glu was the predominant amino acid in all hydrolysates (36–47%), followed by Gly (8–11%), Asp (8–10%), and Arg (5–7%). These findings are consistent with previous studies (Cejudo-Bastante et al., 2022; Mora-Garrido, Escudero-Gilete, Heredia, & Cejudo-Bastante, 2024; Rodríguez-Muñoz et al., 2025). Considering groups, HAA was the most abundant (14–25% of TAA), followed by AAA (4–9% of TAA), and SAA (1–2% of TAA), in all hydrolysates.

Table 3 shows that the type and concentration of the bleaching

Table 2
Nitrogen content peptide (%) and the CIELAB colour parameters (L^*, C^*_{ab}, h_{ab}) of the unbleached and bleached peptides hydrolysates. Mean and standard deviation (n = 3).

	Nitrogen content peptide (%)				Colour parameters (CIELAB units)											
					L^*				C^*_{ab}				h_{ab}			
PH-C	52.21	±	0.54	a	42.36	±	1.44	c	35.18	±	1.52	a	62.45	±	1.82	d
PH-PVPP30	50.25	±	0.65	ab	44.01	±	1.38	c	34.61	±	2.87	a	64.06	±	1.66	d
PH-PVPP150	49.66	±	0.40	b	45.45	±	1.81	c	35.35	±	0.37	a	64.70	±	1.69	d
PH-AC1	51.47	±	0.15	ab	44.79	±	3.34	c	34.24	±	1.87	a	64.92	±	0.98	d
PH-AC5	50.83	±	0.02	ab	55.80	±	4.60	b	22.71	±	1.31	c	79.25	±	1.34	b
PH-R25	42.63	±	1.65	d	46.54	±	7.10	c	34.41	±	2.19	a	62.63	±	3.89	d
PH-R50	46.07	±	0.62	c	62.23	±	1.82	a	29.12	±	1.49	b	70.59	±	2.91	c
PH-AC5+R50	40.63	±	2.06	d	60.29	±	2.42	ab	12.67	±	0.52	d	83.65	±	1.77	a

Soluble peptide hydrolysates (PH) samples: PH-C, control unbleached; PH-PVPP30, treated with 30 g/hL PVPP; PH-PVPP150, treated with 150 g/hL PVPP; PH-AC1, treated with 1 g/L activated charcoal; PH-C5, treated with 5 g/L activated charcoal; PH-R25, treated with 25% resin; PH-R50, treated with 50% resin; PH-C5+R50, treated with 5 g/L activated charcoal + 50% resin. Different letters in the same column indicate significant ($p < 0.05$) differences among samples according to Tukey's test.

product exerted a significant effect on the amino acid content. Thus, PVPP produced no significant differences in the content of total and any individual amino acids. The use of active charcoal had a significant effect in reducing AAA amount, indicating a certain affinity for specific amino acids such as Phe and Tyr. This could be due to the presence of aromatic rings in the structure of these amino acids (Xiao et al., 2014), that favour the electrostatic and dispersive interactions with the surface area of active charcoal (Villacañas et al., 2006). The samples in which resin was applied (PH-R25, PH-R50, and PH-AC5+R50) showed significantly lower content of TAA, and all amino acid families (SAA, HAA, and AAA). Resin bleaching yielded soluble peptide hydrolysates with the lowest TAA values, corresponding to a substantial decrease of 40–64%, mainly due to the loss of HAA and AAA (Ile, Leu, Phe, and Tyr), which was particularly pronounced (Tyr was completely removed) in soluble peptide hydrolysates bleached with sequential use of activated charcoal and resin. According to several recent studies, aromatic amino acids such as Phe and Tyr stabilise anthocyanin compounds by slowing down their degradation process (Chung et al., 2017; Nie et al., 2022). Consequently, the significant decrease in AAA content found in peptides hydrolysates bleached with activated charcoal and resin may restrict their capacity to interact with wine anthocyanins and thus hinder the colour stabilisation. Moreover, the decrease in the amount of specific amino acids seems to be related to the colour changes of bleached peptides hydrolysates. According to Sakai et al. (2022), the interaction between low molecular melanoidins and the amino acids Lys and Arg could be the principal factor that contribute to the protein colour, producing the characteristic brown colour of the soluble peptide hydrolysates. Therefore, the decrease of Lys and Arg (41% and 70% respectively) in peptides hydrolysates treated with resin is consistent with the colour loss (the most bleached hydrolysates) observed (Table 2).

The results indicated that neither TAA or the amino acid families (SAA, HAA, AAA) were significantly influenced ($p > 0.05$) by the concentration used in each bleaching agent. Only punctual significant differences were observed in individual amino acids content: a higher content of Ser and lower of Arg as the concentration of activate charcoal increased (confirming that a high number of interaction points with these amino acids exists), or lower contents of Cys, Ile, Leu, Phe, His, and Arg as resin concentration increased (confirming that higher resin concentration provides more potential interaction sites with these amino acids, facilitating their removal from the soluble peptide hydrolysates).

3.2.4. Antioxidant activity

Fig. 4 shows the antioxidant capacity of all soluble peptide hydrolysates and the effect of the type and concentration of the bleaching agent on antioxidant capacity. The soluble peptide hydrolysates had an inherent high antioxidant capacity, which is consistent with previous results (Rodríguez-Muñoz et al., 2025). The three methods displayed similar trend in all samples.

The type of bleaching product significantly influenced the antioxidant activity. Considering ABTS method, PH-PVPP exhibited the highest values, even higher than control (unbleached), which could be due to the removal of some oxidised phenolic compounds that make increase the total antioxidant capacity of the resulting bleached product. R and AC treatments led to bleached soluble peptide hydrolysates with significantly lower antioxidant capacity, mainly PH-R and PH-AC + R. Results were similar for DPPH and BRS methods.

The amount of bleaching product also affected the antioxidant activity, generally showing inverse concentration-dependent effect. However, these differences were not showed by all the methods. The AC treatment provoked significantly lower values as content increased, observed in this case by the three essays. This effect was displayed only by DPPH assay in PVPP treatments, and in resin treatments by DPPH and ABTS assays.

Taken together, PVPP did not exert negative effects on antioxidant activity, AC was negative only at high concentrations, resin exerted negative effect for all concentrations, and the sequential use of high concentration of AC and resin led to soluble peptide hydrolysates with even lower values, suggesting that these materials remove different antioxidant compounds.

Results from ABTS, DPPH and BRS indicate that resin bleaching, especially when combined with activated charcoal, was associated with a decrease in antioxidant capacity of hydrolysates, which could be related to peptide loss during the bleaching process.

The amino acid composition may also contribute. Pearson analysis supported a direct correlation, for the three methods, between antioxidant activity and concentrations of HAA and AAA, as found in literature (Zhao et al., 2022; Zou et al., 2016). Particularly, for resin treatments, Pearson correlation between concentrations of HAA and AAA, and antioxidant activity by BRS was r = 1. Within the AAA family, Tyr shows strong activity (Liu et al., 2018), thus, the lack of Tyr in PH-AC5+R50 (Table 3) could explain the lowest values of antioxidant activity (Fig. 4).

3.2.5. Peptide composition

The LC–MS/MS analysis was performed on PH-C (control), PH-AC5, PH-R50, and PH-AC5+R50 to determine the amino acid sequences of the peptides in the hydrolysates that exhibited the highest bleaching efficiency and were considered as promising options for potential addition to red wines (Table S1). This analysis allowed a comparative assessment of how each bleaching treatment influenced the peptide sequence of hydrolysates.

The peptides identified in all the hydrolysates ranged from 6 to 22 amino acids and had molecular weights between 604 and 2380 Da. Similar findings have been recently reported in studies of defatted grape seed protein hydrolysates obtained with different enzymes (Mora-Garrido, Escudero-Gilete, Heredia, & Cejudo-Bastante, 2024;

Table 3
Individual and total amino acids content (mg/g hydrolysate) of unbleached and bleached soluble peptide hydrolysates. Mean and standard deviation (n = 3).

	PH-C}				PH-PVPP30}				PH-PVPP150}				PH-AC1}				PH-AC5}				PH-R25}				PH-R50}				PH-AC5+R50}			
Asp	14.32	±	1.06	a	13.51	±	1.32	a	14.12	±	1.07	a	14.12	±	0.57	a	12.95	±	1.17	a	9.62	±	0.09	b	11.53	±	1.78	ab	5.76	±	0.30	c
Thr	4.09	±	0.23	a	3.53	±	0.33	a	3.56	±	0.77	a	3.68	±	0.35	a	4.07	±	0.44	a	2.16	±	0.09	b	2.29	±	0.41	b	1.51	±	0.13	b
Ser	8.37	±	0.34	ab	8.93	±	0.75	a	8.40	±	0.20	ab	8.13	±	0.30	ab	9.10	±	0.43	a	6.37	±	0.08	c	7.53	±	0.86	bc	4.69	±	0.26	d
Glu	61.40	±	4.95	a	59.46	±	3.78	a	58.62	±	5.53	a	61.22	±	4.77	a	63.55	±	3.04	a	40.16	±	0.16	b	53.85	±	9.97	ab	25.84	±	1.26	c
Gly	14.32	±	0.96	ab	14.10	±	0.37	ab	14.04	±	0.80	ab	14.30	±	0.97	ab	14.56	±	0.79	a	9.60	±	0.06	c	11.86	±	1.94	bc	6.75	±	0.29	d
Ala	7.86	±	0.43	a	9.40	±	1.40	a	7.70	±	0.72	a	7.99	±	0.52	a	8.63	±	0.39	cd	5.03	±	0.08	bc	5.72	±	0.96	bc	3.52	±	0.16	d
Cys	1.03	±	0.24	ab	1.01	±	0.05	a	1.04	±	0.11	ab	1.14	±	0.01	ab	1.04	±	0.04	ab	0.78	±	0.03	ab	1.01	±	0.13	ab	0.55	±	0.03	ab
Val	8.09	±	0.62	a	8.17	±	1.39	a	8.04	±	0.70	a	7.91	±	0.67	a	8.17	±	0.38	a	4.79	±	0.08	b	4.38	±	0.86	b	2.18	±	0.16	c
Met	2.79	±	0.80	ab	2.74	±	0.50	a	2.26	±	0.32	abc	2.43	±	0.30	abc	2.67	±	0.18	ab	1.17	±	0.11	bc	1.03	±	0.62	c	0.64	±	0.21	c
Ile	6.19	±	0.51	a	6.25	±	1.04	a	5.84	±	0.40	a	5.97	±	0.18	a	5.40	±	0.38	a	2.62	±	0.04	b	1.72	±	0.32	bc	0.64	±	0.03	c
Leu	9.66	±	0.73	a	9.67	±	0.72	a	9.03	±	0.66	a	9.31	±	0.09	a	8.69	±	0.63	a	3.39	±	0.11	b	2.39	±	0.41	b	0.72	±	0.05	c
Tyr	5.28	±	1.21	a	4.44	±	0.65	ab	4.41	±	0.49	ab	3.87	±	0.54	ab	3.32	±	0.14	b	1.73	±	0.03	c	1.52	±	0.14	cd		nd		
Phe	7.50	±	1.50	a	6.28	±	1.19	ab	6.38	±	0.93	ab	5.91	±	0.80	ab	5.04	±	0.31	b	1.43	±	0.07	c	1.17	±	0.07	c	1.34	±	0.20	c
His	3.00	±	0.14	a	2.82	±	0.21	a	2.84	±	0.21	a	2.78	±	0.17	a	2.45	±	0.03	a	2.15	±	0.03	ab	1.77	±	0.12	b	1.76	±	0.08	b
Lys	3.51	±	0.21	ab	3.33	±	0.25	ab	3.40	±	0.25	ab	3.39	±	0.19	ab	3.26	±	0.04	a	2.68	±	0.03	bc	2.01	±	0.18	c	1.83	±	0.22	c
Arg	11.84	±	0.76	a	12.11	±	1.30	a	12.10	±	0.70	a	11.14	±	0.52	a	9.00	±	0.16	b	7.94	±	0.02	bc	6.06	±	0.81	c	3.53	±	0.15	d
TAA	169.24	±	11.65	a	166.88	±	8.17	a	161.78	±	19.61	a	163.08	±	9.58	a	162.77	±	9.89	a	101.65	±	0.42	b	115.33	±	18.55	b	60.72	±	2.52	c
SAA	4.32	±	0.80	ab	3.75	±	1.96	a	3.30	±	0.43	abc	3.36	±	0.61	abc	3.70	±	0.22	ab	1.94	±	0.13	bc	1.29	±	0.28	bc	1.19	±	0.23	c
HAA	42.09	±	4.08	a	43.32	±	5.42	a	39.25	±	3.74	a	39.51	±	1.95	a	38.60	±	2.24	a	18.44	±	0.24	c	15.89	±	2.99	bc	8.51	±	1.20	c
AAA	15.78	±	2.65	a	13.55	±	1.84	ab	13.63	±	1.63	ab	12.57	±	1.36	ab	11.25	±	1.23	b	5.31	±	0.08	c	4.24	±	0.41	c	2.94	±	0.50	c

Soluble peptide hydrolysates (PH) samples: PH-C, control unbleached; PH-PVPP30, treated with 30 g/hL PVPP; PH-PVPP150, treated with 150 g/hL PVPP; PH-AC1, treated with 1 g/L activated charcoal; PH-C5, treated with 5 g/L activated charcoal; PH-R25, treated with 25% resin; PH-R50, treated with 50% resin; PH-C5+R50, treated with 5 g/L activated charcoal + 50% resin. Amino acids: Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Gly, glycine; Ala, alanine; Cys, cysteine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; His, histidine; Lys, lysine; Arg, arginine; TAA, total amino acids; SAA, sulfur amino acids; AAA aromatic amino acids; HAA hydrophobic amino acids. nd: not detected. Different letters in the same row indicate significant ($p < 0.05$) differences among samples according to Tukey's test.

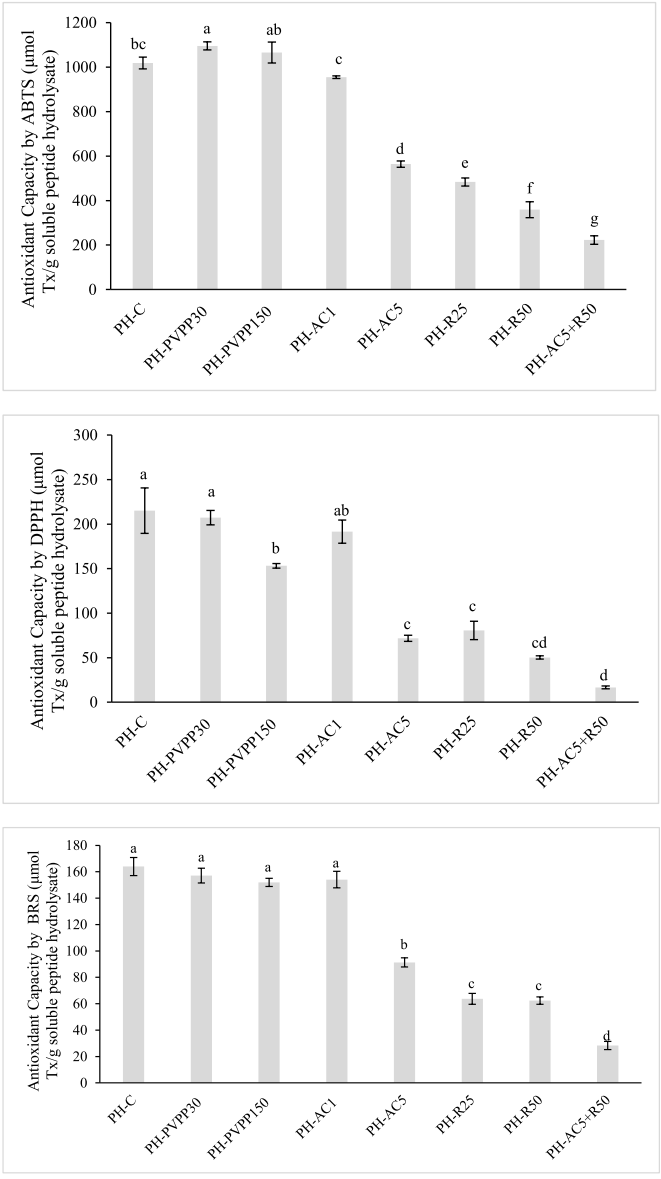

Fig. 4. Antioxidant capacity (µmol Tx/g soluble peptide hydrolysate) measured by ABTS (a), DPPH (b) and BRS (c) of soluble peptide hydrolysates (PH): PH-C, control unbleached; PH-PVPP30, treated with 30 g/hL PVPP; PH-PVPP150, treated with 150 g/hL PVPP; PH-AC1, treated with 1 g/L activated charcoal; PH-C5, treated with 5 g/L activated charcoal; PH-R25, treated with 25% resin; PH-R50, treated with 50% resin; PH-C5+R50, treated with 5 g/L activated charcoal + 50% resin. Bars show mean ± SD (µmol TE/g). For each method, different letters denote significant ($p < 0.05$) differences among samples according to Tukey's test.

Rodríguez-Muñoz et al., 2025). In all samples, over 90% of the peptides were shorter than 15 aa, of which approximately 52–55% were very short chains (<10 aa). Conversely, peptides longer than 20 amino acids constituted less than 1%, not even been detected in PH-AC5+R50.

Only fifty-three proteins from which the identified peptides came were common in all hydrolysates. Some of these proteins (F6HZK2, F6HZK3, A5CL75) were also previously identified in grape seed protein hydrolysates (Mora-Garrido, Escudero-Gilete, Heredia, & Cejudo-Bastante, 2024; Rodríguez-Muñoz et al., 2025). Moreover, peptides derived from the protein D7U302 have been also found in all samples, except in PH-AC5+R50. This is aligned with the number of peptides detected, which considerably decreased after bleaching (Table S1), above all in PH-AC5+R50, affirming that the sequential application of AC and R were more susceptible to remove peptides.

Previous studies have shown that certain amino acids can interact with malvidin-3-O-glucoside wine anthocyanins (Chamizo-González et al., 2021, 2022, 2023). It has also been reported that amino acids at terminal position of peptides are important for antioxidant activity (Zou et al., 2016). Therefore, identifying the residues at N- and C-terminal positions of the peptides is relevant to understand how their addition might influence colour stabilisation and antioxidant properties in red wine. According to Table 4, N-terminal positions of the peptides in unbleached hydrolysates (PH-C) were mainly occupied by negative residues, Glu, Gln, and Val, which is in line with findings in similar peptides hydrolysates (Rodríguez-Muñoz et al., 2025). PH-R50 displayed the same amino acids at N-terminal, affirming that the resin treatment did not change the original distribution of amino acids at N-terminal and, hence, the eventual capability of colour stabilisation of red wines. Furthermore, the treatment with activated charcoal mainly affected Gln, evidencing its affinity for peptides bearing it at that position. The disappearance of Gln at N-terminal positions was in favour to the respective acid (Glu), which has particular interest since malvidin-3-O-glucoside specially binds with Glu (López-Molina et al., 2025), by van der Waals forces and hydrogen bonds, resulting in the formation of copigmentation complexes that contribute to the stabilisation of the anthocyanin. The combined treatment with AC and R led to a similar amino acid profile at N-terminal, although with high contribution of Gly, which has also been proposed to interact with malvidin-3-O-glucoside (Chamizo-González et al., 2023). In this context, the bleached hydrolysates that retain N-terminal Glu (PH-AC5 and PH-R50) and Gly (PH-AC5+R50) could be regarded as having a greater potential to stabilise wine colour by protecting malvidin-3-O-glucoside. These findings are consistent with previous studies about the positive effects of the addition of peptide hydrolysates from defatted grape seed meal with similar amino acids in terminal positions on colour stabilisation of red wines (Mora-Garrido, Escudero-Gilete, González-Miret, et al., 2024, 2026).

Moreover, the predominant amino acids at the C-terminal in the unbleached hydrolysate corresponded to Gln and Leu, followed by Phe (Table 4). These results were in accordance with the findings of Rodríguez-Muñoz et al. (2025). The treatment with activated charcoal (PH-AC5) displayed differences in the C-terminal compared to PH-C, substituting Phe by Arg, which could be related to the significantly lower content of Phe in soluble peptide hydrolysates treated with resin (Table 3). Phe and Arg at the C-terminal position has been demonstrated to enhanced antioxidant activity (Sae-Leaw et al., 2017; Zhu et al., 2024); however, the marked reduction of nitrogen content peptide observed in PH-AC5 and PH-AC5+R50 (Table 2) could mask any potential increase in antioxidant capacity, which is actually lower (Fig. 4), particularly in soluble peptide hydrolysates treated with the combined action of AC and R. The additional use of resin on the soluble peptide hydrolysates treated with activated charcoal (PH-AC5+R50) did not alter the predominant C-terminal amino acid profile, also observed when resin was applied to unbleached soluble peptide hydrolysates. This confirms that bleaching with resin does not modify the distribution of C-terminal amino acids. Moreover, the persistent presence of Gln as C-terminal residue in PH-R50 and PH-AC5+R50 indicates favourable conditions to possible wine colour stabilisation, considering the demonstrated capacity of Gln to interact with malvidin-3-O-glucoside (Chamizo-González et al., 2023).

3.3. PCA

An unsupervised pattern recognition statistical analysis (principal component analysis, PCA) was conducted to identify the principal parameters responsible for the differences between the samples (Fig. 5). Following the Kaiser criterion (eigenvalues > 1), eleven significant principal components were identified, which explain 100% of the total variance. Fig. 5 shows the projection of the samples on the plane defined

Table 4
Distribution (%) of the amino acids at the N-terminal and C-terminal positions of the samples.

	N-terminal			C-terminal		
PH-C	Q (10.0%)	E (9.5%)	V (9.0%)	Q (16.4%)	L (16.2%)	F (13.3%)
PH-AC5	E (13.2%)	N (9.6%)	V (9.6%)	L (18.2%)	Q (13.9%)	R (11.4%)
PH-R50	E (16.1%)	V (10.4%)	Q (8.83%)	Q (19.0%)	L (14.0%)	F (10.4%)
PH-AC5+R50	G (11.8%)	V (10.9%)	N (10.1%)	Q (20.2%)	R (16.8%)	L (16.8%)

Soluble peptide hydrolysates (PH) samples: PH-C, control unbleached; PH-C5, treated with 5 g/L activated charcoal; PH-R50, treated with 50% resin; PH-C5+R50, treated with 5 g/L activated charcoal + 50% resin. Amino acids: N, asparagine; R, arginine; G, glycine; E, glutamic acid; Q, glutamine; L, leucine: F, phenylalanine; S, serine; V, valine.

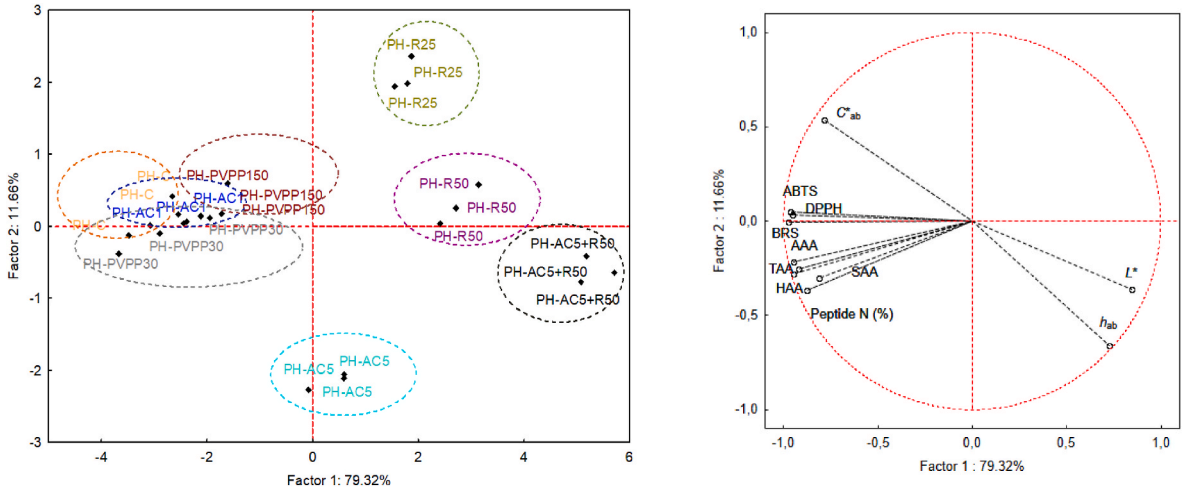

Fig. 5. Scatterplot of soluble peptide hydrolysates (PH) samples (PH-C, control unbleached; PH-PVPP30, treated with 30 g/hL PVPP; PH-PVPP150, treated with 150 g/hL PVPP; PH-AC1, treated with 1 g/L activated charcoal; PH-C5, treated with 5 g/L activated charcoal; PH-R25, treated with 25% resin; PH-R50, treated with 50% resin; PH-C5+R50, treated with 5 g/L activated charcoal + 50% resin) and variables (nitrogen content peptide; TAA, total amino acids; SAA, sulfur amino acids; HAA, hydrophobic amino acids; AAA, aromatic amino acids; antioxidant activity: DPPH, ABTS and BRS; and CIELAB parameters) plotted onto the plane defined by the first two principal components.

by the two principal components (PCs), which explained 90.98% of the total variability. The PC-1 separated by typology of bleached samples that differed the most from the unbleached control soluble peptide hydrolysates. Thus, soluble peptide hydrolysates treated with activated charcoal at 5 g/L (PH-AC5), resin at both concentration (PH-R25 and PH-R50) and the sequential use of bleaching products (PH-AC5+R50) were located in the positive axis, distinguishing from the rest by their higher values of lightness (L^*) and hue (h^*_{ab}), lower values of antioxidant capacity, nitrogen content of peptide and amino acid amounts. PC-2 discriminated according to the concentration of bleaching products, where soluble peptide hydrolysates treated with the highest contents are on the negative axis, mainly associated with lower values of chroma, and higher of lightness and hue, nitrogen content of peptide and all amino acid families (TAA, HAA, SAA, and AAA).

4. Conclusions

The aim of this study was to achieve decolouration (by dephenolisation and further bleaching) of hydrolysates from grape seed meals without compromising the antioxidant peptide fraction, for minimising their colour contribution to be used as colour stabiliser in winemaking. Considering the results, the sequential use of methanol and potassium metabisulphite seems to be the most effective dephenolisation strategy to decolouring the grape seed meal without affecting the peptide fraction. Furthermore, the use of activated charcoal at 5 g/L could be considered the most appropriate option for decolorising grape seed meal soluble peptide hydrolysates, as it effectively reduces colour while preserving peptides and antioxidant amino acids involved in colour stabilisation. In contrast, the additional use of 50% resin produced even more highly decolourised hydrolysates, although assuming a moderate loss of peptide and amino acid content. However, the preservation of terminal amino acids potentially involved in anthocyanin interactions suggesting a possible contribute to colour stabilisation in wine, which could also make them feasible for use in winemaking. The use of PVPP, regardless of concentration, is excluded due to limited decolourising effect. Further research is needed to determinate the effectiveness of the addition of these selected bleached peptides hydrolysates such colour stabiliser of red wine from warm climates, focusing on colour evolution, antioxidant stability, and sensory impact of the resulting wines.

CRediT authorship contribution statement

María del Rosario Rodríguez-Muñoz: Writing – original draft, Investigation. **Fabio Chinnici:** Writing – review & editing, Investigation. **Francisco J. Heredia:** Supervision, Funding acquisition, Conceptualization. **M. Lourdes González-Miret:** Writing – review & editing, Funding acquisition. **María Jesús Cejudo-Bastante:** Writing – review & editing, Writing – original draft.

Funding sources

This publication is part of the project PID2025-171776OB-C22, funded by MICIU/AEI/10.13039/501100011033 and by ERDF/EU.

In addition, the authors thank the project PID2021-127126OB-C22 and the Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED), through the IberBioAl Network (325RT0170).

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge Alvinesa Natural Ingredients S. A. (Daimiel, Ciudad Real, Spain) for supplying the grape seed meal, and Agrovin S.A. (Alcázar de San Juan, Ciudad Real, Spain) for their collaboration and provision of the bleaching agents.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119962.

Data availability

Data will be made available on request.

References

AOAC. (1995). In *Official methods of analysis* (16th ed.). Washington DC: Association of Official Analytical Chemists.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules, 27*(4), 1326. https://doi.org/10.3390/molecules27041326

Bessada, S. M. F., Barreira, J. C. M., & Oliveira, M. B. P. P. (2015). Asteraceae species with most prominent bioactivity and their potential applications: A review. *Industrial Crops and Products, 76*, 604–615. https://doi.org/10.1016/j.indcrop.2015.07.073

Cejudo-Bastante, M. J., Oliva-Sobrado, M., González-Miret, M. L., & Heredia, F. J. (2022). Optimisation of the methodology for obtaining enzymatic protein hydrolysates from an industrial grape seed meal residue. *Food Chemistry, 370*, Article 131078. https://doi.org/10.1016/j.foodchem.2021.131078

Chakka, A. K., & Babu, A. S. (2022). Bioactive compounds of winery by-products: Extraction techniques and their potential health benefits. *Applied Food Research, 2*(1), Article 100058. https://doi.org/10.1016/j.afres.2022.100058

Chamizo-González, F., Estévez, I. G., Gordillo, B., Manjón, E., Escribano-Bailón, M. T., Heredia, F. J., & González-Miret, M. L. (2023). First insights into the binding mechanism and colour effect of the interaction of grape seed 11S globulin with malvidin 3-O-glucoside by fluorescence spectroscopy, differential colorimetry and molecular modelling. *Food Chemistry, 413*, Article 135591. https://doi.org/10.1016/j.foodchem.2023.135591

Chamizo-González, F., Gordillo, B., & Heredia, F. J. (2021). Elucidation of the 3D structure of grape seed 7S globulin and its interaction with malvidin 3-glucoside: A molecular modeling approach. *Food Chemistry, 347*, Article 129014. https://doi.org/10.1016/j.foodchem.2021.129014

Chamizo-González, F., Heredia, F. J., Rodríguez-Pulido, F. J., González-Miret, M. L., & Gordillo, B. (2022). Proteomic and computational characterisation of 11S globulins from grape seed flour by-product and its interaction with malvidin 3-glucoside by molecular docking. *Food Chemistry, 386*, Article 132842. https://doi.org/10.1016/j.foodchem.2022.132842

Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J. (2017). Stability improvement of natural food colors: Impact of amino acid and peptide addition on anthocyanin stability in model beverages. *Food Chemistry, 218*, 277–284. https://doi.org/10.1016/j.foodchem.2016.09.087

CIE. (2004). *Technical report colorimetry; Commission Internationale de l'Éclairage*. Vienna, Austria: Central Bureau.

Corpas, F. J., Rodríguez-Ruiz, M., Campos, M. J., Taboada, J., & Palma, J. M. (2024). Electrochemical detection of total antioxidant capacity (TAC) in plant tissues from different origins. *Methods in Molecular Biology, 2798*, 1–9. https://doi.org/10.1007/978-1-0716-3826-2_1

De Angelis, D., Latrofa, V., Caponio, F., Pasqualone, A., & Summo, C. (2024). Techno-functional properties of dry-fractionated plant-based proteins and application in food product development: A review. *Journal of the Science of Food and Agriculture, 104*(4), 1884–1896. https://doi.org/10.1002/jsfa.13168

De Bruijn, J., Valdebenito, A., Loyola, C., Serra, I., Salazar, F., & López, F. (2009). Continuous stabilization of Chardonnay with ion-exchange resin: Influence on protein and phenolic profiles of wine. *Chilean Journal of Agricultural Research, 69*(1), 54–59. https://doi.org/10.4067/s0718-58392009000100007

Dwyer, K., Hosseinian, F., & Rod, M. (2014). The market potential of grape waste alternatives. *Journal of Food Research, 3*(2), 91. https://doi.org/10.5539/jfr.v3n2p91

El Hosry, L., Elias, V., Chamoun, V., Halawi, M., Cayot, P., Nehme, A., & Bou-Maroun, E. (2025). Maillard reaction: Mechanism, influencing parameters, advantages, disadvantages, and food industrial applications: A review. *Foods, 14*(11), 1881. https://doi.org/10.3390/foods14111881

European Commission. (2023). Proposal for a directive of the European parliament and of the council amending directive 2008/98/EC on waste (COM/2023/420). https://doi.org/10.2760/858144.

Eurostat. (n.d.). Food waste and food waste prevention – Estimates. Statistics Explained. Retrieved from https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Food_waste_and_food_waste_prevention_-_estimates. Accessed August 4, 2025.

Firdaous, L., Fertin, B., Khelissa, O., Dhainaut, M., Nedjar, N., Chataigné, G., Ouhoud, L., Lutin, F., & Dhulster, P. (2017). Adsorptive removal of polyphenols from an alfalfa white protein concentrate: Adsorbent screening, adsorption kinetics and equilibrium study. *Separation and Purification Technology, 178*, 29–39. https://doi.org/10.1016/j.seppur.2017.01.009

Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R., & Pintado, M. (2021). Valorisation of food agro-industrial by-products: From the past to the present and perspectives. *Journal of Environmental Management, 299*, Article 113571. https://doi.org/10.1016/j.jenvman.2021.113571

Hernández-Rodríguez, P., Baquero, L. P., & Larrota, H. R. (2019). *Flavonoids: Potential therapeutic agents by their antioxidant capacity. Bioactive compounds: Health benefits and potential applications*. Elsevier. https://doi.org/10.1016/B978-0-12-814774-0.00014-1

Jara-Palacios, M. J., Gonçalves, S., Heredia, F. J., Hernanz, D., & Romano, A. (2020). Extraction of antioxidants from winemaking byproducts: Effect of the solvent on phenolic composition, antioxidant and anti-cholinesterase activities, and electrochemical behaviour. *Antioxidants, 9*(8), 675. https://doi.org/10.3390/antiox9080675

Jara-Palacios, M. J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-Gilete, M. L., & Heredia, F. J. (2014). Detailed phenolic composition of white grape by-products by RRLC/MS and measurement of the antioxidant activity. *Talanta, 125*, 51–57. https://doi.org/10.1016/j.talanta.2014.02.065

Jiang, J., Nie, Y., Sun, X., & Xiong, Y. L. (2021). Partial removal of phenolics coupled with alkaline ph shift improves canola protein interfacial properties and emulsion in in vitro digestibility. *Foods, 10*(6). https://doi.org/10.3390/foods10061283

Liu, J., Zhang, D., Zhu, Y., Wang, Y., He, S., & Zhang, T. (2018). Enhancing the in vitro antioxidant capacities via the interaction of amino acids. *Emirates Journal of Food and Agriculture, 30*(3), 224–231. https://doi.org/10.9755/ejfa.2018.v30.i3.1641

López-Molina, M. F., Rodríguez-Pulido, F. J., Mora-Garrido, A. B., González-Miret, M. L., & Heredia, F. J. (2025). New approaches for screening grape seed peptides as colourimetric modulators by malvidin-3-O-glucoside stabilisation. *Food Chemistry, 464*, Article 141708. https://doi.org/10.1016/j.foodchem.2024.141708

Lorenc-Grabowska, E. (2015). Effect of micropore size distribution on phenol adsorption on steam activated carbons. *Adsorption, 22*(4–6), 599–607. https://doi.org/10.1007/s10450-015-9737-x

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International, 43*(7), 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Mora-Garrido, A. B., Cejudo-Bastante, M. J., Heredia, F. J., & Escudero-Gilete, M. L. (2022). Revalorization of residues from the industrial exhaustion of grape by-products. *LWT – Food Science and Technology, 156*, Article 113057. https://doi.org/10.1016/j.lwt.2021.113057

Mora-Garrido, A. B., Escudero-Gilete, M. L., González-Miret, M. L., Heredia, F. J., & Cejudo-Bastante, M. J. (2024). Effect of the addition of protein hydrolysates from grape seed meal residue to red wines in warm regions in stabilization stage. *LWT – Food Science and Technology, 205*, Article 116554. https://doi.org/10.1016/j.lwt.2024.116554

Mora-Garrido, A. B., Escudero-Gilete, M. L., González-Miret, M. L., Heredia, F. J., & Cejudo-Bastante, M. J. (2026). Using <5 kDa peptide fraction from grape seed by-product as colour stabiliser of red wines in warm climates. *LWT-Food Science and Technology, 249*, Article 119409. https://doi.org/10.1016/j.lwt.2026.119409

Mora-Garrido, A. B., Escudero-Gilete, M. L., Heredia, F. J., & Cejudo-Bastante, M. J. (2024). Enzymatic protein hydrolysates of a residue from grape by-products industry for winemaking application: Influence of the starting material and hydrolysis time. *Cogent Food & Agriculture, 10*(1), Article 2314231. https://doi.org/10.1080/23311932.2024.2314231

Narváez-Cuenca, C. E., Kuijpers, T. F. M., Vincken, J. P., De Waard, P., & Gruppen, H. (2011). New insights into an ancient antibrowning agent: Formation of sulfophenolics in sodium hydrogen sulfite-treated potato extracts. *Journal of Agricultural and Food Chemistry, 59*(18), 10247–10255. https://doi.org/10.1021/JF202624Q

Nie, M., Wang, L., Lu, S., Wang, Y., Zheng, M., & Fang, Z. (2022). Protective effect of amino acids on the stability of bayberry anthocyanins and the interaction mechanism between l-methionine and cyanidin-3-O-glycoside. *Food Chemistry, 396*, Article 133689. https://doi.org/10.1016/j.foodchem.2022.133689

Pismenskaya, N., Sarapulova, V., Klevtsova, A., Mikhaylin, S., & Bazinet, L. (2020). Adsorption of anthocyanins by cation and anion exchange resins with aromatic and aliphatic polymer matrices. *International Journal of Molecular Sciences, 21*(21), 7874. https://doi.org/10.3390/ijms21217874

Postai, A. N., Muxel, A. A., Zapp, E., & Brondani, P. B. (2025). Enzymatic modification of polyvinylpolypyrrolidone (PVPP) for improved adsorption of proteins and polyphenols causing haze in beer. *ACS Food Science & Technology, 5*(2), 843–850. https://doi.org/10.1021/acsfoodscitech.4c01004

Rdsa, L. D. L. D., Santana, M. C., Avezedd, T. L., Brígoda, A. O. S., Gdddy, R., Pachecd, S., Mellonger-Solva, C., & Cabral, L. M. C. (2018). A comparison of dual-functional whey hydrolysates by the use of commercial proteases. *Food Science and Technology, 38*, 31–36. https://doi.org/10.1590/fst.08417

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rodríguez-Muñoz, M.d. R., Mora-Garrido, A. B., Heredia, F. J., Cejudo-Bastante, M. J., & González-Miret, M. L. (2025). Obtention and characterisation of antioxidant-rich

peptides from defatted grape seed meal using different enzymes. *Foods, 14*(7), 1248. https://doi.org/10.3390/foods14071248/s1

Ruggieri, L., Cadena, E., Martínez-Blanco, J., Gasol, C. M., Rieradevall, J., Gabarrell, X., Gea, T., Sort, X., & Sánchez, A. (2009). Recovery of organic wastes in the Spanish wine industry. Technical, economic and environmental analyses of the composting process. *Journal of Cleaner Production, 17*(9), 830–838. https://doi.org/10.1016/j.jclepro.2008.12.005

Sae-Leaw, T., Karnjanapratum, S., O'Callaghan, Y. C., O'Keeffe, M. B., FitzGerald, R. J., O'Brien, N. M., & Benjakul, S. (2017). Purification and identification of antioxidant peptides from gelatin hydrolysate of seabass skin. *Journal of Food Biochemistry, 41*(3), Article 12350. https://doi.org/10.1111/jfbc.12350

Sakai, K., Okada, M., & Yamaguchi, S. (2022). Decolorization and detoxication of plant-based proteins using hydrogen peroxide and catalase. *Scientific Reports, 12*(1), 1–10. https://doi.org/10.1038/s41598-022-26883-8

Schieber, A. (2018). Reactions of quinones - Mechanisms, structures, and prospects for food research. *Journal of Agricultural and Food Chemistry, 66*(50), 13051–13055. https://doi.org/10.1021/acs.jafc.8b05215

Siebert, K. J., & Lynn, P. Y. (2007). On the mechanisms of adsorbent interactions with haze-active proteins and polyphenols. *Journal of the American Society of Brewing Chemists, 66*(1), 48–54. https://doi.org/10.1094/asbcj-2007-1116-02

Simões, S., Costa, A., Faria-Silva, A. C., Ascenso, A., Marto, J., Carvalheiro, M., Gonçalves, L. M., Marques, M., Paiva, A., Bento, M., Simões, P., & Ribeiro, H. M. (2021). Sustainable valorization of food-processing industry by-products: Challenges and opportunities to obtain bioactive compounds. *Valorization of agri-food wastes and by-products: Recent trends, innovations and sustainability challenges*. Elsevier. https://doi.org/10.1016/B978-0-12-824044-1.00023-4

Singh, R., Langyan, S., Sangwan, S., Rohtagi, B., Khandelwal, A., & Shrivastava, M. (2022). Protein for human consumption from oilseed cakes: A review. *Frontiers in Sustainable Food Systems, 6*, Article 856401. https://doi.org/10.3389/fsufs.2022.856401/bibtex

Soler-Rivas, C., Espín, J. C., & Wichers, H. J. (2000). An easy and fast test to compare total free radical scavenger capacity of foodstuffs. *Phytochemical Analysis, 11*(5), 330–338. https://doi.org/10.1002/1099-1565(200009/10)11:5<330:AID-PCA534>3.0.CO;2-G

Tilley, A., McHenry, M. P., McHenry, J. A., Solah, V., & Bayliss, K. (2023). Enzymatic browning: The role of substrates in polyphenol oxidase mediated browning. *Food Science, 7*, Article 100623. https://doi.org/10.1016/j.crfs.2023.100623

Villacañas, F., Pereira, M. F. R., Órfão, J. J. M., & Figueiredo, J. L. (2006). Adsorption of simple aromatic compounds on activated carbons. *Journal of Colloid and Interface Science, 293*(1), 128–136. https://doi.org/10.1016/J.JCIS.2005.06.032

Wang, X., Liu, H., He, H., Luo, X., Yang, H., Bhullar, K. S., Chen, B., Su, D., Zhao, T., & Zhou, W. (2025). Optimizing decolorization and deodorization to remove pigments and fishy odors while preserving antioxidant activity in Asian swamp eel (*Monopterus albus*) hydrolysate. *Food Chemistry X, 28*, Article 102545. https://doi.org/10.1016/j.fochx.2025.102545

Xiao, C., Zheng, L., Su, G., & Zhao, M. (2014). Effect of solution pH and activated carbon dosage on the decolourization ability, nitrogen components and antioxidant activity of peanut meal hydrolysate. *International Journal of Food Science and Technology, 49*(12), 2571–2577. https://doi.org/10.1111/ijfs.12587

Yu, K., He, W., Ma, X., Zhang, Q., Chen, C., Li, P., & Wu, D. (2024). Purification and biochemical characterization of polyphenol oxidase extracted from wheat bran. *Molecules, 29*(6), 1334. https://doi.org/10.3390/molecules29061334

Zhao, F., Liu, C., Bordoni, L., Petracci, I., Wu, D., Fang, L., Wang, J., Wang, X., Gabbianelli, R., & Min, W. (2022). Advances on the antioxidant peptides from nuts: A narrow review. *Antioxidants, 11*(10), 2020. https://doi.org/10.3390/antiox11102020

Zhu, Z., Xu, Z., Li, Y., Fan, Y., Zhou, Y., Song, K., & Meng, L. (2024). Antioxidant function and application of plant-derived peptides. *Antioxidants, 13*(10), 1203. https://doi.org/10.3390/antiox13101203

Zou, T. B., He, T. P., Li, H. B., Tang, H. W., & Xia, E. Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules, 21*(1), 72. https://doi.org/10.3390/molecules21010072