

***Eucalyptus globulus* Leaf Extract as a Natural Alternative to Sulphites in Red Wine Stabilization**

Ana Silva

LSRE-LCM, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
(up202004518@up.pt) ORCID [0009-0008-2934-5260](https://orcid.org/0009-0008-2934-5260)

Catarina Abreu

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202500514@up.pt) ORCID [0009-0006-9488-3204](https://orcid.org/0009-0006-9488-3204)

Inês Madeira

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202004190@up.pt) ORCID [0009-0007-6661-9497](https://orcid.org/0009-0007-6661-9497)

Maria Caldas

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202108357@up.pt) ORCID [0009-0006-1437-4726](https://orcid.org/0009-0006-1437-4726)

Nikolas Carvalho

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202102373@up.pt) ORCID [0009-0004-8551-7726](https://orcid.org/0009-0004-8551-7726)

Tim Rediestra

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202006585@up.pt) ORCID [0009-0003-9541-7940](https://orcid.org/0009-0003-9541-7940)

Tomás Caldas

Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal (up202107833@up.pt) ORCID [0009-0001-2600-1322](https://orcid.org/0009-0001-2600-1322)

Priscila Corrêa

LEPABE, ALiCE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 PORTO, Portugal
(pscorrea@fe.up.pt) ORCID [0000-0003-1490-6594](https://orcid.org/0000-0003-1490-6594)

Author Keywords

Eucalyptus Globulus, Eucalyptol, Wine, Sulphites, Antioxidant Activity, Circular Economy.

Type: Research Article

 Open Access

 Peer Reviewed

 CC BY

Abstract

Rising environmental concerns and health awareness among consumers are influencing the fermented beverage market. This work proposes *Eucavinum*, a wine in which sulphites are replaced by extracts from *Eucalyptus globulus* leaves derived from agro-industrial waste streams. Process simulation predicts an annual production of 2 million bottles, targeting 150 mg/L of eucalyptol for its antimicrobial and potential antioxidant activity. Preliminary laboratory validation through hydroalcoholic Soxhlet extraction achieved an extraction yield of $(24.6 \pm 1.2) \%$ and a eucalyptol concentration of 200 $\mu\text{g/L}$. Antioxidant characterization revealed total phenolic contents of 118.0–131.2 $\text{mg}_{\text{GAE}}/\text{g}_{\text{extract}}$ and moderate radical scavenging activity (ABTS and DPPH IC_{50} : 86.0 and 141.3–157.2 mg/L , respectively). A preliminary economic assessment indicated the potential feasibility of the proposed process, highlighting the valorization of eucalyptus residues as a promising natural alternative to sulphites in wine stabilization.

1. Introduction

The global beverage market is undergoing a significant shift, driven by a modern consumer base that increasingly prioritizes health, sustainability, and clean-label transparency. Growing

public awareness of nutritional value and health implications of beverages has affected purchasing behaviors (Bazzani et al. 2019).

The Portuguese wine sector demonstrates a strong consumer loyalty and consistent growth, with red wine being one of the most consumed varieties, particularly in the northern region of the country (Santana et al. 2021). Despite this, there has been an increasing demand for natural wines, defined as low-intervention products without processing aids or additives (Vecchio et al. 2021). This evolving preference presents a challenge for the red wine industry, which must align consumers' expectations for a traditional, authentic sensory experience with rigorous technical requirements to ensure product stability (Ballester et al. 2024).

A common additive is sulphur dioxide (SO₂), which has long been regarded as indispensable in winemaking for its antioxidant and antiseptic properties, essential for preserving wine quality and stability (Giacosa et al. 2019; Guerrero and Cantos-Villar 2015). Nevertheless, its use has become increasingly regulated due to its association with health risks (Guerrero and Cantos-Villar 2015).

Eucalyptus species are fast-growing plants and highly adaptable to harsh environmental conditions. Due to their rapid growth and resistance, they can compete with native vegetation for water and nutrients, potentially posing a threat to certain ecosystems. They are currently used in the wood industry for pulp, timber, and paper production (Álvarez et al. 2021; Gelaye and Getachew 2026). *Eucalyptus* leaves are often regarded as an abundant by-product. However, they are a rich source of bioactive compounds, including eucalyptol and other terpenoids, which are recognized for their antioxidant and antimicrobial properties. (Immaroh et al. 2021).

This work aims to address limitations associated with red wine production while delivering a sustainable, authentic and health-conscious product by substituting SO₂ with *Eucalyptus Globulus* leaf extracts.

2. State of the Art

According to the International Organisation of Vine and Wine (2025) Portugal is currently the 10th top wine producer. From 2014 to 2024, the increase in wine production in Portugal was from 6.2 million hL to 6.9 million hL, accompanied by an increase in consumption per capita (48.2 L to 61.1 L) and a 5% increase in exportation. In 2024, 50% of the produced wine was exported (International Organisation of Vine and Wine 2025). The global wine market was valued at \$398 million in 2024 expected to grow at a compound annual growth rate (CAGR) of 4.03% (DataBridge 2025).

Red wine is essentially a hydroalcoholic solution produced from the fermentation of grape must. Its production also requires a maceration step to extract phenolic compounds, such as tannins and anthocyanins, which determine the wine's color, aroma, structure and aging potential (Morata et al. 2019). After fermentation and maceration, red wines are turbid and unstable, containing a mixture of microorganisms (yeast and bacteria), tartrate crystals, grape skin and pulp cell debris, and molecular aggregates that cause hazes and deposits. Clarification processes, such as filtration, removes these particles, improving limpidity and reducing microbial load. Nonetheless, further stabilization is needed to preserve wine quality and prevent microorganism proliferation from bottling to consumption, regardless of storage or transport conditions (Vernhet 2019).

Sulphur dioxide and its sulphite salts (E220-E228) are preservatives traditionally added to wine to prevent oxidation and microbiological spoilage (Fia et al. 2023; Stockley et al. 2021). SO₂ reacts with oxygen in a series of redox reactions - conversion to sulphite and then to sulphate -

preventing oxidation of sensitive compounds. The reduced availability of oxygen limits the growth of aerobic microorganisms (Stockley et al. 2021). Despite their widespread use and preservative benefits, sulphites have been associated with health risks, including anaphylactic reactions, asthmatic exacerbation, and other symptoms such as dermatitis, urticaria, flushing, hypotension, abdominal pain, and diarrhea (Vally and Misso 2012). From an oenological perspective, sulphites can also impact the sensory qualities of the wine or even produce characteristic aroma defects (Guerrero and Cantos-Villar 2015).

As a result, the reduction or removal of sulphites has become a key focus in the wine industry. Under European Union regulations, wines containing more than 10 mg/L of sulphites must carry a label statement “contains sulphites”. In Europe, the legal limit for total SO₂ in red wines is set at 150 mg/L. The World Health Organization recommends a maximum daily intake of 0.7 mg/kg body weight, which for an individual weighing 60–80 kg corresponds to 42–56 mg per day, an amount easily exceeded by consuming even 0.5 L of wine at the maximum permitted sulphite level (Giacosa et al. 2019).

As previously mentioned, Eucalyptus species are fast-growing trees capable of surviving in harsh environmental conditions. Their vigorous growth and strong competition for water and nutrients can pose ecological challenges in certain regions. According to the 6th Forest National Inventory (Instituto da Conservação da Natureza e das Florestas 2019), the most abundant species in Portugal is *Eucalyptus globulus*, covering 845,000 ha, which represents 26% of the Portuguese forest area (Malico et al. 2021). Economically, they are widely used for the wood industry, particularly for pulp, timber, and paper production. Meanwhile, bark, leaves, and branches are by-products (Álvarez et al. 2021; Gelaye and Getachew 2026). From the Eucalyptus wood industries' by-products, essential oils with high antioxidant, anti-bacterial, and anti-inflammatory properties can be extracted. Leaf extract has been highly used as an aroma, flavor and natural preservative in the food sector (Immaroh et al. 2021).

Eucalyptus leaf extracts show promise as a natural alternative preservative to SO₂. It is mainly composed by oxygenated monoterpenes (1,8-cineole, α -terpineol, terpinene-4-ol, linalool), monoterpenes (α -pinene, β -pinene) or sesquiterpene (α -eudesmol, globulol, epiglobulol), flavonoids, phenolic acids and tannins (Immaroh et al. 2021; Bencheikh et al. 2021).

According to the European Medicines Agency (2013), it is mainly composed by 1,8-cineole (eucalyptol) in an amount of 54-95%. This component is known for its antimicrobial and anti-inflammatory activity. Other compounds such as α -pinene, β -pinene, limonene, β -myrcene, p-cymene, terpineol and globulol have antioxidant properties (Harkat-Madouri et al. 2015; Surbhi et al. 2021).

This paper focuses on the addition of *E. globulus* leaves extract to a natural (additives-free) red wine, as an alternative to sulphites.

The viability of *E. globulus* essential oil as a preservative has been investigated by Kačániová et al. (2024), who evaluated its potential use as a natural food preservative and demonstrated the strong antibacterial activity of 1,8-cineole. proving the high potential of 1,8-cineole against bacteria for its high antibacterial activity. This project was inspired by the work of González-Rompinelli et al. (2013), who reported the first winery-scale trial involving the addition of antimicrobial plant extracts during wine ageing in wood.

3. Chemical Product Design (CPD)

Before producing the final product, a thorough evaluation of various innovative ideas was conducted. This process followed the methodology outlined in *Chemical Product Design* by Cussler and Moggridge (2011) and previously applied by Oliveira et al. (2024). In line with this

framework, market needs were first identified and translated into design requirements – *Needs and Ideas*. Subsequently, a wide range of potential product ideas was systematically assessed and narrowed down to a single most promising solution – *Selection*. The steps described above were followed rigorously and are detailed in the following sections.

3.1. Needs and Ideas

Initially, a comprehensive cross-sectoral analysis was performed to identify broad requirements on the fermented beverages market. Following this assessment, an ideation phase generated widespread concepts designed to address the identified gaps. To ensure market viability and commercial success, understanding consumer preferences and industry trends is essential. The fermented beverages sector exhibits four primary market needs that guide product development.

The demand for health-conscious and functional products is growing, reflecting the adoption of a wellness-oriented lifestyle. Consumers increasingly seek beverages that offer benefits beyond basic refreshment, such as digestive health improvements through probiotics, enhanced nutrition with added proteins, or reduced alcohol content for healthier consumption alternatives (Gupta et al. 2023). Allied with the health-oriented perspective, the awareness of environmental sustainability and natural products is expanding, with an increasing demand for complete or partial substitution of synthetic chemical additives with natural, bio-based ingredients derived from agro-industrial waste streams, promoting circular economy principles through waste valorization (Gupta et al. 2023). In addition, sensory innovation has also been of particular interest, mainly regarding aroma and taste profiles. Consumers are typically attracted to novel and distinctive flavor combinations, promoting the development of beverages featuring unconventional or exotic aromatic notes designed to stimulate curiosity and enhance overall sensory experience (Mestre Furlani et al. 2024). Finally, the preservation of local tradition and authenticity remains an important market driver. This trend supports the incorporation of regional raw materials and use of traditional techniques that reinforce cultural identity (Fernández-Ferrín et al. 2018). It is important to consider that consumers prioritize practical and convenient solutions that integrate seamlessly into modern lifestyles, favoring ready-to-drink formats and products with extended shelf life.

Taking into account the identified market trends, different product ideas were conceived. The proposed product ideas include a function-targeted kombucha beverage enriched with probiotic strains derived from the fermentation of agro-industrial residues, such as fruit pomace hydrolysates; a beer enhanced with soluble protein to increase nutritional value; and the replacement of sulphites in red wine with *Eucalyptus* leaves. Additionally, a protein-enriched plant-based kefir is formulated as a vegan alternative with improved nutritional content. Another concept involves the development of a fermented beverage from agro-industrial residues through the conversion of fruit peels and pomace into a value-added drink. Finally, it is proposed the extraction of anthocyanins from fruits (grapes, blackberries or blueberries), which exhibits pH- and light-sensitive to create a fermented beverage with photosensitive coloring (Khoo et al. 2017).

3.2. Selection

Following the identification of needs and ideas, a systematic evaluation of the conceptualized products was conducted. Each product was scored on a scale from 1 to 5 according to predefined criteria, weighted as follows: innovation and market differentiation (10%), assessed based on the number of articles retrieved from Scopus (2026) using descriptive

keywords related to each concept; impact on beverage's flavor (10%); product engineering, including technical feasibility and process complexity (20%); environmental impact and promotion of circular economy (20%); market demand and fulfillment of the previously identified needs (15%); market competition with other products and brands (5%); and final cost of production (20%). **Table 1** presents the selection matrix with the scored for the top three product ideas. The complete evaluation matrix and the detailed justification for the assigned scores are provided in **Supplementary Information in Table A.1**.

Regarding the three highest-performing concepts, a considerable score difference can be observed for the kombucha with probiotics derived from agro-industrial residues and protein beer. For the first idea, this is mainly due to its low demand and innovation. Conventional kombucha already has probiotics, and probiotics obtention from agro-wastes showed already some research. Additionally, the sourcing from waste may require heavy regulation and approval for health-claims. Relative to a protein-enriched beer, engineering feasibility is limited, since protein addition may lead to haze formation, turbidity and instability, requiring complex stabilization or clarification processes. Competition with big brands and increased cost due to protein stabilization treatments highly influence its position in the market. The increase demand for an alternative to sulphites and simple extraction of bioactive compounds from plant extracts led to a higher score for the remaining idea. Therefore, the incorporation of Eucalyptus leaves extract in red wine as an SO₂ replacement showed a superior performance across multiple criteria. It has a notably high innovation potential, strong sustainability performance and significant market demand. Its moderately low-cost rating reflects the established availability of *E. globulus* within Portugal's agro-industrial sector, facilitating economically viable large-scale production. Furthermore, this approach directly addresses identified market needs for natural ingredients and chemical-free preservation systems, aligning with European regulatory trends toward reduced synthetic additives in fermented beverages.

Product idea	Innovation (10%)	Flavor impact (10%)	Engineering (20%)	Sustainability (20%)	Demand (15%)	Competition (5%)	Cost (20%)	Total
Kombucha with probiotics agro-industrial residues	3	4	2	5	2	3	3	3.2
Protein beer	5	3	2	3	3	2	3	3.0
Replacement of SO ₂ in red wine with Eucalyptus leaves extracts	4	3	5	5	5	3	4	4.4

Table 1: Selection matrix with criteria and respective scores for the top 3 product ideas.

4. Product

Eucavinum is an innovative red wine free of artificial sulphites produced from the *Vinhão* grape variety sourced from Adega Vila Real in Portugal's Douro region. The *Vinhão* red wine base is characterized by its natural acidity and robust tannin profiles. This product uses hydroalcoholic extraction of *E. globulus* bioactive compounds, with special focus on

eucalyptol, which is then incorporated in *Eucavinum* as a natural stabilizer. Our product presents an alcohol content of 13% v/v, pH of 3.4, dry classification with low residual sugars, and a fruity aromatic profile. It contains a small amount of naturally occurring sulphites (around 15 mg/L). This is due to SO₂ production by yeast during fermentation, meaning a minimal level of SO₂ is always present (Stockley et al. 2021). Theoretically, *Eucavinum* contains 150 mg/L of eucalyptol.

After fermentation, *Eucavinum* is aged in oak barrels at 13°C, allowing the integration of eucalyptus extracts while developing the robust polyphenol and tannin structure characteristic of premium Douro red wines. The following work had as guideline the studies of González-Rompinelli et al. (2013).

5. Manufacture

The manufacturing process of *Eucavinum* will be done in batches and can be divided into three main stages. The first is eucalyptus leaves pretreatment, which includes washing, drying and grinding of the leaves. Besides enhancing the extraction process, this step allows the preservation and storage of the raw material. Subsequently, eucalyptus oil – with special focus on eucalyptol – will be extracted using a hydroalcoholic solvent, which will then be evaporated for pure extract obtainment. Finally, the extract is incorporated into the red wine, aged for 6 months and packaged.

The untreated red wine is supplied by Adega Vila Real. The closeness to one of the main suppliers led to the establishment of the *Eucavinum* production facility in Vila Real. Eucalyptus globulus leaves are provided as by-products by the company Navigator. Bottles, labels, and corks for wine storage and branding are purchased from PORTOdoVIDRO, who will also be responsible for the sales of *Eucavinum*.

6. Process Simulation

The following section details the simulation of the manufacture process in the software *SuperPro Designer* version 13 (Intelligen 2023). All input and output streams and final mass balance are reported in **Supplementary Information** in **Table B.1** and **Figure B.1**, respectively.

6.1. Leaf Pretreatment and Preservation

Post arrival from Navigator, all leaves would undergo a pretreatment process to preserve them. This procedure would be carried out once per year. To do so, a feed stream of 2.2 ton/batch *E. globulus* leaves, with a relative humidity (H_r) of 50 %, was washed (WSH-101), with a total of 5 m³/batch of water. A recycling loop of the water was set with a filter to remove any debris from the raw material. Washed leaves ($H_r=80\%$) were then dried in five tray dryers (TDR-101) for 6 h, reducing moisture to around 20%. The selected drying temperature was 60°C to avoid any degradation of the bioactive compounds. Subsequently, biomass particles size was reduced to 0.2 mm through a grinding step (GR-101) to increase contact surface area between the solvent and the leaves, thus enhancing extraction efficiency. Following this treatment, it is assumed that the resulting biomass (1.4 ton/batch) would be stored in cold, hermetic storage, using 100 kg plastic bags. Process flow diagram is presented in **Figure 1**.

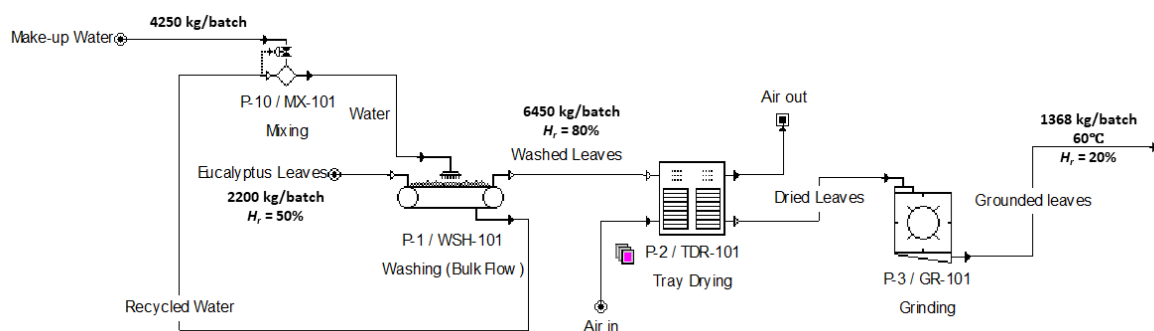


Figure 1: Flow diagram of SuperPro Designer simulation of the pretreatment of *E. globulus* leaves.

6.2. Hydroalcoholic Extraction Process

The following step involves the bioactive extraction from the ground leaves, as illustrated in **Figure 2**. The biomass and solvent, ethanol 40% (v/v), were fed to a solid-liquid extractor (MSX-101), which was simulated as a mixer-settler unit due to model limitations. The extraction was modeled at 75°C, assuming an extraction yield of 27%, considering the optimized yields reported by Álvarez et al. (2021) for extractions performed with ethanol or water as solvents. The dissolved extract is separated from the leaves. The top stream of the extractor is then filtered (SP-101) to completely remove any residual biomass. The filtrate follows to a multi-effect evaporator (EV-101) for 6 h at 40°C and vacuum (0.09 bar) to recover the solvent, which is then treated for reuse. A complete separation of the extract from the solvent was assumed in this step. The final eucalyptus extract stream has a total mass flow of 374 kg/batch with the final composition presented in **Table 2**.

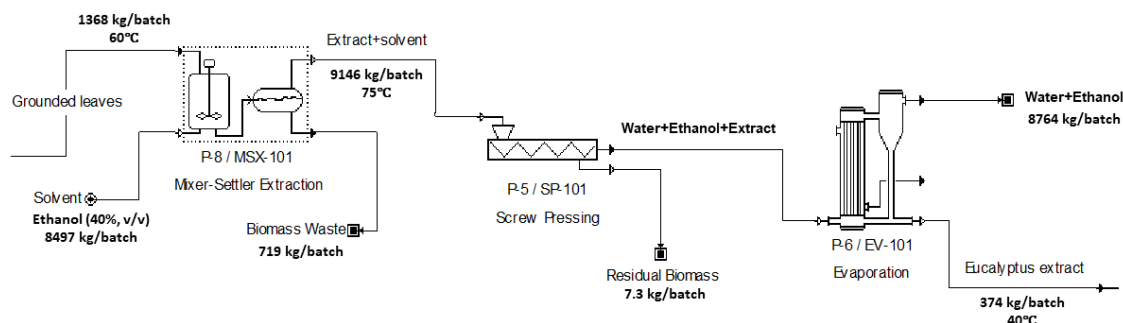


Figure 2: Flow diagram of SuperPro Designer simulation of the extraction process.

Compound	α -pinene	Aromadendrene	1,8-cineole	Globulol	Limonene	Viridiflorol
Composition / %	5.9	5.9	58.8	17.6	5.9	5.9

Table 2: Composition of the final 'Eucalyptus extract' stream.

6.3. Eucalyptus Extract Incorporation into Wine

The final manufacturing step consists of mixing the eucalyptus extract with the purchased wine. The red wine (1500 m³) is delivered untreated and has already completed alcoholic fermentation, hence only the addition of the extract (374 kg) is required. This process is modeled in three mixers, which in practice would correspond to 10 steel barrels with 10 m³ capacity.

Eucavinum post-mixing stream possesses a final concentration of eucalyptol 150 mg/L. Post extract incorporation, the wine is stored in 225 L oak barrels for 6 months at a temperature range of 13-18°C. Finally, the aged *Eucavinum* is filled in 0.75 L bottles and labeled. A total of around 2 million bottles is obtained per year.

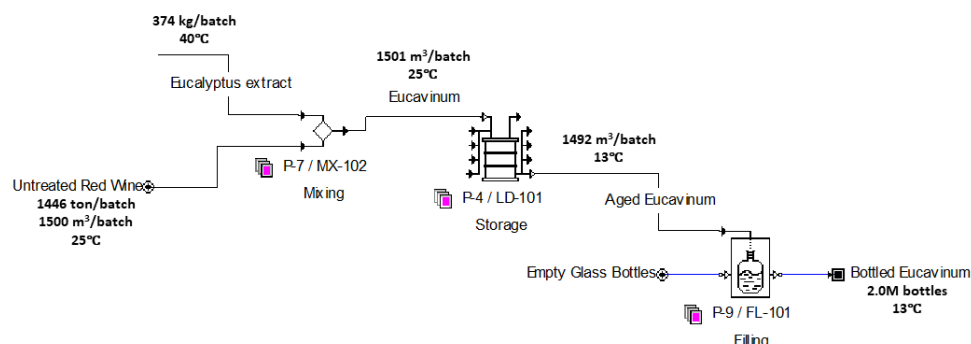


Figure 3: Flow diagram of SuperPro Designer simulation of extract incorporation into wine.

7. Laboratory Validation

For process validation, preliminary assays were executed at lab-scale, including leaves pretreatment, eucalyptol hydroalcoholic extraction, solvent evaporation, extract characterization and wine incorporation.

7.1. Materials

HPLC-grade ethanol (CAS 64-17-5, ≥99.9%) was purchased from Sigma Aldrich (Darmstadt, Germany). For characterization, 1,8-cineole (CAS 470-82-6, 99%) from Alfa Aesar (Kandel, Germany). *Eucalyptus globulus* leaves were collected from a mature tree in Gondomar, Portugal.

7.2. Leaves Pretreatment

To evaluate the moisture content of the eucalyptus leaves, 30 g of leaves were dried in an oven at 60°C for 4 h. The relative humidity was determined by comparing the mass loss during drying to the initial mass of the leaves, yielding a value of 3.8% (w/w). Subsequently, the dried biomass was ground for further use.

7.3. Hydroalcoholic Extraction and Solvent Recovery

Two extraction methods - Soxhlet (SOX) and ultrasound-assisted extraction (UAE) – were evaluated, both using 150 mL ethanol-water mixture (40:60 % v/v) as solvent and 25 g of shredded leaves. The Soxhlet extraction was performed for 4 h. UAE was performed using a Qsonica Q700 Sonicator (Connecticut, USA) fitted with a specialized probe. The biomass and solvent were placed in a beaker and sonicated in pulse mode (4 min on and 2 min off) for 1 h applied at 60% amplitude. The mixture was then filtered to separate the extract from the plant waste. For both methods, the solvent was removed from the extract using a rotary evaporator. The extractions were performed in duplicate.

Total extraction yield (Y_{total}) was determined through **Formula (1)**, where m_{extract} represents the mass of extract weighted after solvent evaporation, and m_{leaves} the initial mass of the leaves.

$$Y_{\text{total}} = \frac{m_{\text{extract}}}{m_{\text{leaves}}} \cdot 100 \quad (1)$$

7.4. Chemical Characterization of the Extract

Quantitative and qualitative chemical composition of the Soxhlet extracts was evaluated. Gas chromatography coupled with mass spectrometry (GC-MS) was performed using a Shimadzu GCMS-TQ8040 NX Triple Quadrupole (Kyoto, Japan) equipped with a splitless injector and a cross bonded fused column (30 m x 0.25 mm, 0.25 µm film thickness). The oven temperature was programmed in multiple sequential ramps, as described by Kessler et al. (2025). Initially, it was set to isothermal at 40°C for 1 min, followed by heating to 200°C at 7°C/min rate, held for 2 min. Then, at a rate of 15°C/min, it increased to 250°C for 2 min and, finally, to 280°C for 1 min at 20°C/min. Helium was used as carrier gas at a constant flow rate of 1 mL/min. The injector, ion source and interface temperatures were set to 290°C, 250°C and 260°C, respectively. For analysis, samples of 1 µL were injected.

A calibration curve was established using standard solutions of eucalyptol ranging from 1 to 100 mg/L ($Y = 2.1 \times 10^9 X + 9.8 \times 10^6$, $R^2 = 0.9920$), allowing the correlation between GC peak area and eucalyptol concentration to be determined. The extract was redispersed in absolute ethanol at a known concentration and filtered through a hydrophilic PES membrane filter (0.45 µm pore size).

The yield of eucalyptol extracted ($Y_{\text{eucalyptol}}$) and its concentration in the extract ($c_{\text{eucalyptol}}$) were calculated through **Formulas (2) and (3)**, respectively, where $m_{\text{eucalyptol}}$ represents the mass of eucalyptol extracted.

$$Y_{\text{eucalyptol}} = \frac{m_{\text{eucalyptol}}}{m_{\text{leaves}}} \cdot 100 \quad (2)$$

$$c_{\text{eucalyptol}} = \frac{m_{\text{eucalyptol}}}{m_{\text{extract}}} \cdot 100 \quad (3)$$

7.5. Extract Antioxidant Activity

7.5.1. Total Phenolic Content (TPC) – Folin-Ciocalteu Assay

The Folin-Ciocalteu assay was performed according to Ferreira et al. (2023). Briefly, 20 µL of the diluted sample (1000 mg/L), 100 µL of Folin-Ciocalteu reagent and 1580 µL of distilled water were mixed and kept in the dark. After 3-6 min, 300 µL of saturated sodium carbonate solution (333.3 g/L) was added. The absorbances were read at 750 nm after 2 h of reaction. Distilled water was used as blank and gallic acid was used as a standard. The results were expressed as mg_{GAE}/g_{extract} through a correlation curve using 50 to 1000 mg/mL of gallic acid solutions ($Y = 0.1007 X - 0.0632$, $R^2 = 0.9964$).

7.5.2. ABTS Scavenging Activity

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity of the extracts was evaluated according to Xiao et al. (2020). To do so, 12-16 h before analysis, an ABTS reactive solution was prepared by mixing 1:1 (v/v) of ABTS solution (3.84 mg/mL) and potassium persulphate solution (0.66 mg/mL), both prepared in 0.05M sodium acetate buffer (pH=4.6). The reactive solution was then diluted (1:50, v/v) in the acetate buffer and kept in the dark for 30 min. Afterwards, 180 µL of ABTS reactive solution was added to 20 µL of the sample, and the absorbance at 734 nm was read after 15 min of reaction. Acetate buffer was used as the control and replacing the reactive solution in the blank. The percentage of radical

inhibition was calculated in relation to the control, and the IC_{50} values were expressed as Trolox equivalents per gram of extract ($mg_{TE}/g_{extract}$). The extracts were analyzed in a range of 195.3-20,000 $\mu g/mL$ as previously described by Park et al. (2023).

7.5.3. DPPH Scavenging Activity

To assess the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity, 180 μL of DPPH ethanolic solution (150 $\mu mol/L$) was added to 20 μL of the diluted sample. The resulting solution was kept in the dark for 40 min, and the absorbance measured at 515 nm. Distilled water was used as control and blank, and absolute ethanol replaced the DPPH solution in the blank (Bobo-García et al. 2015). Similarly to the ABTS analysis, the extracts were analyzed in the same concentration range, and both the percentage of radical inhibition and the IC_{50} values ($mg_{TE}/g_{extract}$) were calculated.

7.6. Wine Incorporation

To achieve a final volume of 750 mL, 24.4 mL of extract (28.64 $g_{extract}/L$) was incorporated into 725.6 mL of red wine.

7.7. Results and Discussion

7.7.1. Extraction Efficiency and Extract Composition

Higher extraction yields were obtained for Soxhlet extraction (24.62 ± 1.22) % compared to UAE (9.91 ± 0.18) %. These results are consistent with the literature. Rodrigues et al. (2018) reported a 25.90% yield for Soxhlet extraction using absolute ethanol as solvent. Álvarez et al. (2021) achieved yields of 30.8-38.7 wt.% with ethanol and 22.3-35.2 wt.% with water.

For UAE, Lima et al. (2025) attained 8.34-25.05% of extraction yield with ethanol (50% v/v), depending on extraction time and power. This suggests that the UAE procedure should be further optimized in the future. Due to the low extraction yield (<10%) obtained with UAE, only the extracts produced by Soxhlet solid-liquid extraction were characterized in terms of chemical composition and antioxidant activity.

The results for both Soxhlet assays (SOX_1 and SOX_2) are present in **Table 3**. A considerable difference between SOX_1 and SOX_2 highlights the need for additional replicates to assess reproducibility.

	SOX_1	SOX_2
$Y_{eucalyptol} / \%$	0.0024	0.0048
$C_{eucalyptol} / \%$	0.0096	0.022

Table 3: Eucalyptol extraction yields and mass concentration in the extract.

The composition of the extract was analyzed, and the identified compounds along with their relative abundances are presented in **Table 4**. A total of 21 components were identified. According to literature, eucalyptol typically constitutes for 50-80% of *E. globulus* leaves extract, although some lower values (10-20%) have been reported (Surbhi et al. 2021; Barbosa et al. 2016). This is mainly due to the its variability with geographic origin and environmental conditions (Surbhi et al. 2021). Nonetheless, as shown in **Table 3** and **Table 4**, the concentration of eucalyptol is considerably lower than the expected range (58.8% from simulation). This discrepancy may be associated with a non-optimized extraction process, including parameters such as extraction time, solvent ratio and temperature. Additionally, during rotary evaporation, some volatile components like eucalyptol may have been partially lost along with the solvent due to their high volatility.

The relative composition of eucalyptol reported in **Table 4** differs significantly from its concentration in the extract present in **Table 3** because GC-MS relative composition is calculated as a normalized value based solely on the detected volatile compounds, excluding non-volatile components. In contrast, the concentration determined using the calibration curve reflects eucalyptol's proportion in the total extract.

Identified Components	Retention Time / min	Relative Concentration / %
D-limonene	7.59	0.80
1,8-cineole	7.64	1.29
α -terpineol acetate	13.85	3.08
(+)-aromadendrene	15.57	9.19
(-)-alloaromadendrene	15.95	2.29
Germacrene	16.59	0.89
Epiglobulol	17.75	1.88
Globulol	18.16	11.02
(-)-caryophyllene	18.29	2.56
Elemol	18.78	2.46
Myrcenol	18.91	4.21
Methyl α -D-glucopyranoside	19.26	22.26
Viridiflorol	20.49	0.69
Isopulegol acetate	20.55	1.29
L-(+)-Ascorbic acid 2,6-dihexadecanoate	23.90	2.24
Phytol	26.28	5.22
Linolenyl alcohol	26.77	2.80
exo-2-Hydroxycineole	29.35	4.96
α -tocopherol- β -D-mannoside	32.56	12.71
Spinacen	33.09	8.16

Table 4: Identified compounds from GC-MS analysis of *E.globulus* extract.

Regarding the remaining components present in the extract, although their relative percentages differ from previous studies, the identities are consistent with literature on *E. globulus* leaf extracts (Immaroh et al. 2021). As mentioned, its chemical profile varies significantly depending on extraction method, geographic location, and environmental conditions. For instance, Nigussie and Werede (2020) reported that the main components in the leaves of *E. globulus* extracts were 1,8-cineole (68–72%), cis-sabinol (12–14%), limonene (2–3%), α -pinene (2–3%), and α -terpineol (1–2%). Meanwhile, Song et al. (2009) identified 1,8-cineole (72%), α -pinene (9.22%), α -terpineol (2.54%), (-)-globulol (2.77%), α -terpineol acetate (3.11%), and alloaromadendrene (2.47%).

Even if eucalyptol content is relatively low, other components have well-documented antioxidant and antimicrobial activities. These include phytol, α -tocopherol derivatives (α -tocopherol- β -D-mannoside) (Gololo et al. 2016), ascorbic acid derivatives (and L-(+)-ascorbic acid 2,6-dihexadecanoate) (Panihar et al. 2018), viridiflorol, globulol and germacrene (Pripdeevech and Chukeatirote 2010).

7.7.2. Antioxidant Activity – TPC, ABTS, DPPH

As shown in **Table 5**, SOX₁ and SOX₂ presented 131.2 and 118.0 mg_{GAE}/g_{extract} of total phenolic content, respectively. These results are in accordance with those reported in the literature. For instance, a study on methanolic extracts from thirteen different eucalyptus species cultivated in North East of Tunisia have reported a wide variation in content among species, ranging from 16.6 to 122.8 mg_{GAE}/g_{DW}. Interestingly, the polyphenol content in *E. globulus* was about 20 mg_{GAE}/g_{DW} (Limam et al. 2021), far lower than those obtained in this study. However, other authors have reported higher contents in *E. globulus* leaf extracts, reaching 235.9 mg_{GAE}/g_{DW} (Dezsi et al. 2015) and ranging from 126.7 to 497.7 mg_{GAE}/g_{DW} through extraction at different ethanol concentrations (Park et al. 2023). Surprisingly, the IC₅₀ values for DPPH radical scavenging in the latter study (188.2 to 5841.7 mg/L) were higher than those observed in the present study (141.3 and 157.2 mg/L). This means that, despite having a higher TPC, higher concentrations of these extracts are required to achieve 50% radical inhibition than SOX₁ and SOX₂. Regarding the activity against ABTS radicals, SOX₁ presented 51.6% of inhibition at the lowest concentration tested (19.4 mg/L), thus it was not possible to calculate the IC₅₀ in the tested conditions. Accordingly, SOX₂ presented low IC₅₀ value (86.0 mg/L). On the other hand, the Trolox equivalents found in the extracts (SOX₁ and SOX₂) were quite low for both radicals (≤ 32.5 mg_{trolox}/g_{extract}). Indeed, regardless of species or cultivation conditions, the extraction method and solvent selected play a crucial role in determining the antioxidant capacity of plant extracts. In agreement with this, the findings of González-Burgos et al. (2018) for *E. globulus* leaf extracts showed that compounds extracted using acetone and methanol as solvents exhibited a stronger ability to inhibit the ABTS radical compared with those extracted with ethanol. In contrast, for the DPPH assay, the differences among solvents were less pronounced, indicating that the antioxidants responsible for DPPH inhibition are similarly extracted by all three solvents.

Antioxidant properties		SOX ₁	SOX ₂
TPC	mg _{GAE} /g _{extract}	131.2 ± 1.1	118.0 ± 7.3
	IC ₅₀ (mg/L)	141.3 ± 2.1	157.2 ± 1.0
DPPH	TE (mg _{trolox} /g _{extract})	31.0 ± 0.5	27.9 ± 0.2
	IC ₅₀ (mg/L)	*	86.0 ± 5.9
ABTS	TE (mg _{trolox} /g _{extract})	*	32.5 ± 2.4
	IC ₅₀ (mg/L)	*	

Table 5: Antioxidant properties of the Eucalytus extracts. ABTS: 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-Diphenyl-1-picrylhydrazyl, GAE: gallic acid equivalent, TE: Trolox equivalent, TPC: total phenolic content. *The percentage of radical inhibition was greater than 50% at the lowest concentration tested (19.4 mg/L).

7.8. Eucavinum Final Evaluation

The final eucalyptol concentration in *Eucavinum* was 200 µg/L, significantly lower than the expected 150 mg/L predicted by SuperPro Designer simulation, which also led to reduced antioxidant activity. These results highlight the need to optimize extraction conditions to increase both the overall extract yield and, in particular, eucalyptol content. Further investigation into the extract composition using *E. globulus* leaves from the intended supplier, Navigator, is also necessary, as leaf source may influence the chemical profile. Additionally, future studies should assess antimicrobial activity to fully evaluate the feasibility of substituting SO₂ with the extract.

8. Economic Analyses

An economic analysis was employed to verify the profitability and viability of the project, considering a 5-year return period. **Figure 4** contains the Gantt chart showing the project involvement for the first 5 years of activity, including research and development (R&D), marketing, engineering (equipment installation and start-up), production and sales. As wine production is seasonal and concludes in September, all remaining operations were scheduled accordingly. Leaves would be collected from Navigator, pre-treated, and stored once per year. Production would only start in the fourth trimester of the first year. As *Eucavinum* is aged for 6 months, sales would only begin in the second trimester of the second year of activity.

As mentioned, the manufacturing plant of *Eucavinum* will be based in Vila Real, for its closeness to the red Wine supplier – Adega Vila Real. The cost of the company's manufacturing plant, including land, building, consumables, water and energy, would be around €701 thousand. Equipment costs, including installation and piping, amounted to an initial expense of €2 million. Relative to marketing and R&D, 150 bottles would be used for the last and 4850 bottles for marketing purposes, such as advertising, wine tasting events and public relations (PR) packages. This amounts to a total annual cost of €8.7 thousand for the first 10 trimesters of activity, with marketing costs being cut to half for the remaining years.

The business is expected to function 250 days a year, 8 h per day. The production would last around 7 h per day, with one extra hour for start-up of the factory or washing of the equipment at the end of the day.

	1 st Year				2 nd Year				3 rd Year				4 th Year				5 th Year			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
R&D																				
Marketing																				
Engineering																				
Production																				
Sales																				

Figure 4: Gantt chart of the project for 5 years of activity.

8.1. Production Costs

The total costs of the main raw materials and manufacturing process are presented in **Table 6**, accounting for an annual production of 2 million *Eucavinum* bottles. The biomass is assumed to be supplied by Navigator as a by-product; thus, no costs were associated with it other than transportation. It was assumed that the cost of transportation of *Eucavinum* wine is covered by PORTODOSVIDROS, while the project covers the transportation of empty bottles, corkscrews, and labels to the manufacturing facility. Ethanol supply cost includes both the make-up ethanol required to compensate for losses and its recycling for reuse. Utilities and water consumption costs were obtained from *SuperPro Designer*. In terms of labor, the assumptions include 11 employees covering management, administrative, commercial and operational positions. Salary expenses including benefits and insurances led to a total of €253 thousand per year. Therefore, the production of *Eucavinum* amounts to a total cost of 2.99€ per bottle.

	Cost per 100 bottles (€/100 bottles)
Raw Material	
Red wine untreated	75.41
Bottles, cork stoppers and labels	67.91
Transportation	0.71
Ethanol (w/recycling)	0.38
Manufacture	
Water	0.43
Electricity	112.30
Steam	29.60
Labor	
Employee salaries (w/benefits and insurance)	12.74
Total Production Cost	299.48

Table 6: Raw materials and production costs.

8.2. Sales Volume and Product Cost

To ensure a profit margin of 35% relative to the total production cost, *Eucavinum* would be sold at 4.6€ per bottle. Besides the prepared bottles for marketing, a complete sale of the produces wine is expected, i.e., 1.98 million bottles. As previously mentioned, the sales will be made to PORTOdosVIDROS, who is responsible for the selling and distribution.

8.3. Financial Results

The project's financial evaluation indicates strong economic viability. At a 15% discount rate, the Net Present Value (NPV) is 2.1 M€, meaning that the project is expected to generate more value than the initial investment (2.8 M€). The Return on Investment (ROI), calculated as the ratio of NPV to the initial investment, is 75%, reflecting the project's true profitability over time.

Figure 5a) illustrates the accumulated costs and revenues over six years, highlighting the point at which revenues exceed costs and demonstrating long-term economic sustainability. **Figure 5b)** presents the yearly cash flow and cumulative cash flow throughout the project duration. The initial negative cash flow is due to upfront investment and early operational costs with no source of revenue. As sales begin in year 2, cash flow turns positive, allowing the project to recover the initial investment in approximately 4.4 years.

The internal rate of return (IRR) is 30%, exceeding the discount rate and confirming project attractiveness. The profitability index (PI) of 1.75 meaning that each euro invested returns €1.75 in present value terms.

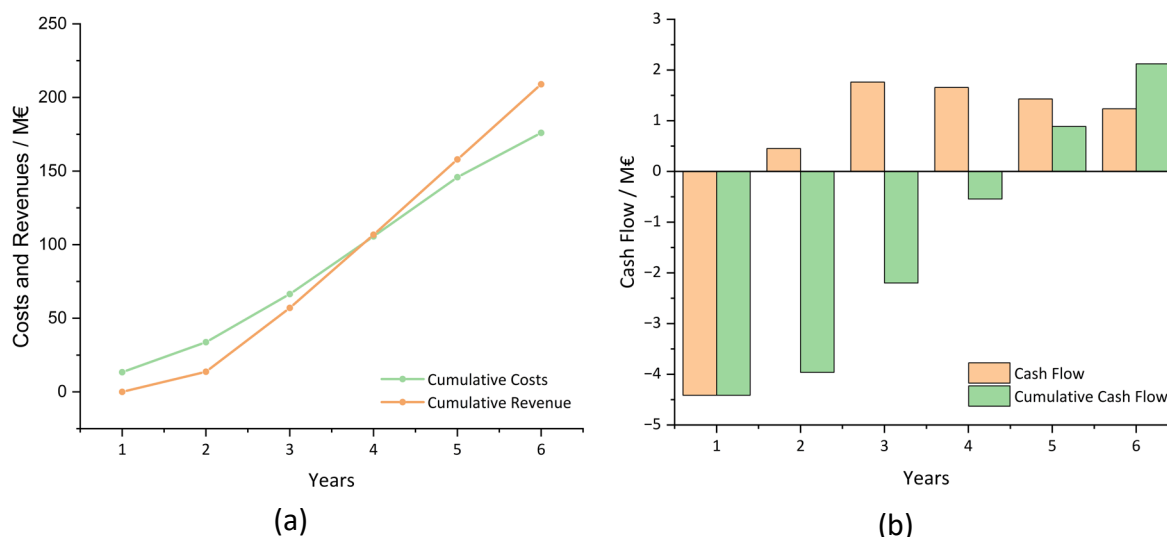


Figure 5: (a) Predicted cumulative costs and revenues and (b) cash-flow and cumulative cash-flow during the six years of the project.

9. Conclusion

This study proposes the production process and product design of *Eucavinum*, a wine in which sulphites are replaced by extracts from *E. globulus* leaf. The extract, and in particular the compound eucalyptol, acts as a potential natural stabilizer due to its antimicrobial and antioxidant activities. Besides developing a product that addresses the need to substitute potentially harmful additives such as SO₂, this project also promotes sustainable practices and circular economy principles by valorizing agro-industrial waste streams.

The proposed production process is divided into three main stages: *E. globulus* leaves pretreatment and preservation, hydroalcoholic extraction followed by solvent recovery, and incorporation of the extract into additive-free red wine. Process simulation using *SuperPro Designer* predicted an annual production capacity of approximately 2 million *Eucavinum* bottles, assuming an expected eucalyptol concentration of 150 mg/L obtained from 2.2 ton/batch of leaves.

A preliminary laboratory validation was conducted through hydroalcoholic Soxhlet extraction, achieving an extraction yield of (24.62 ± 1.22) % and a maximum eucalyptol concentration of 0.022% in the extract. This resulted in a final eucalyptol concentration of 200 µg/L in the produced wine. The extracts showed moderate total phenolic content (118.0–131.2 mg_{GAE}/g_{extract}) and antioxidant activity when compared to literature, with DPPH IC₅₀ values of 141.3–157.2 mg/L and Trolox equivalents of 27.9–32.5 mg_{Trolox}/g_{extract}. In the ABTS assay, SOX₂ presented an IC₅₀ of 86.0 mg/L, while SOX₁ achieved over 50% inhibition at the lowest tested concentration (19.4 mg/L).

Nevertheless, the significantly lower eucalyptol concentration obtained experimentally compared with the simulated value highlights the need for further investigation. Future studies should focus on increasing the number of experimental replicates and optimizing the extraction protocol to improve the recovery of target compounds. These improvements are expected to enhance the reliability of the experimental data and improve the consistency between laboratory results and simulated production, thereby providing a more accurate assessment of the process's efficiency and scalability.

Finally, an economic analysis was performed to evaluate the viability of the project. With a selling price of *Eucavinum* at 4.60€/bottle, the NPV of the project is 2.1 M€, leading to an IRR

of 30% and payback time of 4.4 years. The positive NPV and reasonable payback time highlight the feasibility of the project.

References

- Álvarez, Xana, Ángeles Cancela, Yolanda Merchán *et al.* 2021. "Anthocyanins, Phenolic Compounds, and Antioxidants from Extractions of Six Eucalyptus Species." *Applied Sciences* 2021, Vol. 11, Page 9818 11 (21)<https://doi.org/10.3390/app11219818>.
- Ballester, Jordi, Mirian Belascoín, Marivel Gonzalez-Hernandez, Eva Parga-Dans, Pablo Alonso González, and Maria-Pilar Saenz-Navajas. 2024. "Sensory Attributes and Quality Perception of Red Natural Wines: A Comparative Study in Spain and France." *OENO One* 58 (1)<https://doi.org/10.20870/oeno-one.2024.58.1.7737>.
- Barbosa, Luiz Claudio Almeida, Claudinei Andrade Filomeno, Robson Ricardo Teixeira, Luiz Claudio Almeida Barbosa, Claudinei Andrade Filomeno, and Robson Ricardo Teixeira. 2016. "Chemical Variability and Biological Activities of Eucalyptus Spp. Essential Oils." *Molecules* 2016, Vol. 21, Page 1671 21 (12)<https://doi.org/10.3390/molecules21121671>.
- Bazzani, Claudia, Roberta Capitello, Elena Claire Ricci *et al.* 2019. "Nutritional Knowledge and Health Consciousness: Do They Affect Consumer Wine Choices? Evidence from a Survey in Italy." *Nutrients* 2020, Vol. 12, Page 84 12 (1)<https://doi.org/10.3390/nu12010084>.
- Bencheikh, Dalila, Ahlam Gueddah, Khadidja Soualat *et al.* 2021. "Polyphenolic Contents, Antioxidant and Antibacterial Activities of Aqueous Extracts of Eucalyptus Globulus L. And Trigonella Foenum-Greacum L." *Journal of Applied Biological Sciences* 15 (1):53–63. <https://www.jabsonline.org/index.php/jabs/article/view/833>
- Bobo-García, Gloria, Gabriel Davidov-Pardo, Cristina Arroqui, Paloma Vírseda, María R Marín-Arroyo, and Montserrat Navarro. 2015. "Intra-Laboratory Validation of Microplate Methods for Total Phenolic Content and Antioxidant Activity on Polyphenolic Extracts, and Comparison with Conventional Spectrophotometric Methods." *Journal of the Science of Food and Agriculture* 95 (1)<https://doi.org/10.1002/jsfa.6706>.
- Cussler, E. L., and G. D. Moggridge. 2011. *Chemical Product Design*.
- DataBridge. 2025. "Global Wine Market Size, Share, and Trends Analysis Report – Industry Overview and Forecast to 2032." <https://www.databridgemarketresearch.com/reports/global-wine-market>.
- Dezsi, Ștefan, Alexandru S. Bădăraș, Cristina Bischin *et al.* 2015. "Antimicrobial and Antioxidant Activities and Phenolic Profile of Eucalyptus Globulus Labill. And Corymbia Ficifolia (F. Muell.) K.D. Hill & L.A.S. Johnson Leaves." *Molecules* 20 (3):4720–34. <https://doi.org/10.3390/molecules20034720>.
- European Medicines Agency, EMA. 2013. "Assessment Report on Eucalyptus Globulus Labill., Folium."
- Fernández-Ferrín, Pilar, Aitor Calvo-Turrientes, Belén Bande, Miren Artaraz-Miñón, and M. Mercedes Galán-Ladero. 2018. "The Valuation and Purchase of Food Products That Combine Local, Regional and Traditional Features: The Influence of Consumer Ethnocentrism." *Food Quality and Preference* 64 <https://doi.org/10.1016/j.foodqual.2017.09.015>.
- Ferreira, Sara M., Sandra M. Gomes, Lúcia Santos, Sara M. Ferreira, Sandra M. Gomes, and Lúcia Santos. 2023. "The Chemistry Behind Biological Properties of Agro-Industrial

- Portuguese by-Products." *Waste and Biomass Valorization* 2023 15:5 15 (5)<https://doi.org/10.1007/s12649-023-02366-3>.
- Fia, Giovanna, Silvio Menghini, Eleonora Mari *et al.* 2023. "Replacement of So₂ with an Unripe Grape Extract and Chitosan during Oak Aging: Case Study of a Sangiovese Wine." *Antioxidants* 2023, Vol. 12, Page 365 12 (2)<https://doi.org/10.3390/antiox12020365>.
- Gelaye, Yohannes, and Kiros Getachew. 2026. "Environmental Impacts, Land-Use Trade-Offs, and Sustainable Management Pathways of Eucalyptus Plantation Expansion in Ethiopia." *The Scientific World Journal* 2026 (1)<https://doi.org/10.1155/tswj/6629432>.
- Giacosa, Simone, Susana Segade, Enzo Cagnasso, Alberto Caudana, Luca Rolle, and Vincenzo Gerbi. 2019. "So₂ in Wines: Rational Use and Possible Alternatives." In. <https://doi.org/10.1016/B978-0-12-814399-5.00021-9>.
- Gololo, Stanley, Ntwanano Mapfumari, Lesibana Samuel Sethoga, Mutendela Olivier, Leshweni or Leshwene Shai, and Motetelo Mogale. 2016. "Identification of Phytochemical Constituents within the N-Hexane Leaf Extract of Senna Italica (Mill) Using Gas Chromatography-Mass Spectrometry (Gc-Ms) Analysis." *Journal of Pharmaceutical Sciences and Research* 8:1141–43.
- González-Burgos, Elena, Mindaugas Liaudanskas, Jonas Viškelis, Vaidotas Žvikas, Valdimaras Janulis, and M. Pilar Gómez-Serranillos. 2018. "Antioxidant Activity, Neuroprotective Properties and Bioactive Constituents Analysis of Varying Polarity Extracts from Eucalyptus Globulus Leaves." *Journal of Food and Drug Analysis* 26 (4)<https://doi.org/10.1016/j.jfda.2018.05.010>.
- González-Rompinelli, Eva M., Juan José Rodríguez-Bencomo, Almudena García-Ruiz *et al.* 2013. "A Winery-Scale Trial of the Use of Antimicrobial Plant Phenolic Extracts as Preservatives during Wine Ageing in Barrels." *Food Control* 33 (2)<https://doi.org/10.1016/j.foodcont.2013.03.026>.
- Guerrero, Raúl F., and Emma Cantos-Villar. 2015. "Demonstrating the Efficiency of Sulphur Dioxide Replacements in Wine: A Parameter Review." *Trends in Food Science & Technology* 42 (1)<https://doi.org/10.1016/j.tifs.2014.11.004>.
- Gupta, Achala, Nikita Sanwal, Mohammed A. Bareen *et al.* 2023. "Trends in Functional Beverages: Functional Ingredients, Processing Technologies, Stability, Health Benefits, and Consumer Perspective." *Food Research International* 170 <https://doi.org/10.1016/j.foodres.2023.113046>.
- Harkat-Madouri, Lila, Boudria Asma, Khodir Madani *et al.* 2015. "Chemical Composition, Antibacterial and Antioxidant Activities of Essential Oil of Eucalyptus Globulus from Algeria." *Industrial Crops and Products* 78 <https://doi.org/10.1016/j.indcrop.2015.10.015>.
- Immaroh, N Z, D E Kuliahsari, S D Nugraheni, N Z Immaroh, D E Kuliahsari, and S D Nugraheni. 2021. "Review: Eucalyptus Globulus Essential Oil Extraction Method." *IOP Conference Series: Earth and Environmental Science* 733 (1)<https://doi.org/10.1088/1755-1315/733/1/012103>.
- Instituto da Conservação da Natureza e das Florestas, I.P. 2019. "6º Inventário Florestal Nacional."
- Intelligen, Inc. 2023. "Superpro Designer."
- International Organisation of Vine and Wine, OIV. 2025. *Country Report - Portugal*.

- Kačániová, Miroslava, Natália Čmíková, Nenad L. Vukovic *et al.* 2024. "Eucalyptus Globulus Essential Oil as Food Preservative: Chemical Composition, Antimicrobial (in Vitro and in Situ), Antibiofilm and Insecticidal Potential." *South African Journal of Botany* 175 <https://doi.org/10.1016/j.sajb.2024.10.022>.
- Kessler, Júlia Cristiê, Isabel M. Martins, Yaidelin A. Manrique *et al.* 2025. "Microencapsulated A-Tocopherol and Moringa Extract for Improved Skin Protection: Insights from Human Skin Assessment in Cosmetic Formulations." *Journal of Cosmetic Dermatology* 24 (10)<https://doi.org/10.1111/jocd.70486>.
- Khoo, Hock Eng, Azrina Azlan, Sou Teng Tang, and See Meng Lim. 2017. "Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits." *Food & Nutrition Research* 61 (1)<https://doi.org/10.1080/16546628.2017.1361779>.
- Lima, Laíres, Ana I. Pereira, Clara B. Vaz *et al.* 2025. "Optimization of Heat and Ultrasound-Assisted Extraction of Eucalyptus Globulus Leaves Reveals Strong Antioxidant and Antimicrobial Properties." *Food Chemistry* 479 <https://doi.org/10.1016/j.foodchem.2025.143755>.
- Limam, Hajer, Yosra Ezzine, Sonia Tammar *et al.* 2021. "Phenolic Composition and Antioxidant Activities of Thirteen Eucalyptus Species Cultivated in North East of Tunisia." *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 155 (3):587–97. <https://doi.org/10.1080/11263504.2020.1762791>.
- Malico, Isabel, Ana Cristina Gonçalves, Isabel Malico, and Ana Cristina Gonçalves. 2021. "Eucalyptus Globulus Coppices in Portugal: Influence of Site and Percentage of Residues Collected for Energy." *Sustainability* 2021, Vol. 13, Page 5775 13 (11)<https://doi.org/10.3390/su13115775>.
- Mestre Furlani, María V., Mercedes F. Vargas Perucca, Diego B. Petrignani *et al.* 2024. "Enhancing Flavor Complexity in Craft Beer: Sequential Inoculation with Indigenous Non-Saccharomyces and Commercial Saccharomyces Yeasts." *Fermentation* 10 (12):657. <https://doi.org/10.3390/fermentation10120657>.
- Morata, Antonio, Carmen González, Wendu Tesfaye, Iris Loira, and Jose A. Suárez-Lepe. 2019. "Maceration and Fermentation: New Technologies to Increase Extraction." In *Red Wine Technology*. <https://doi.org/10.1016/B978-0-12-814399-5.00003-7>.
- Nigussie, Gashaw, and Yemane Werede. 2020. "Chemical Profile and Antimicrobial Activity of Essential Oil of Eucalyptus Globulus Leaves from Haramaya Campus and Entoto Park, Ethiopia 1." 6:187–98.
- Oliveira, Ricardo A., Ana Luísa S. Silva, Joana P. Ramos, and Maria Luísa S. Pereira. 2024. "Resinut: A Bio Phenol Formaldehyde Resin Using Chestnut Shells." *U.Porto Journal of Engineering* 10 (2)https://doi.org/10.24840/2183-6493_0010-002_002650.
- Panihar, Neeraj, Shreya Sethi, and Neeru Vasudeva. 2018. "Preliminary Study of Antioxidant Potential and Gas Chromatography-Mass Spectroscopy (Gc-Ms) Analysis of Brassica Oleracea Florets." *International Journal of Green Pharmacy (IJGP)* 12 (02)<https://doi.org/10.22377/ijgp.v12i02.1831>.
- Park, Jae Y., Ju Y. Kim, Yun G. Son *et al.* 2023. "Characterization of Chemical Composition and Antioxidant Activity of Eucalyptus Globulus Leaves under Different Extraction Conditions." *Applied Sciences* 13 (17):9984. <https://doi.org/10.3390/app13179984>.
- Pripdeevech, Patcharee, and Ekachai Chukeatirote. 2010. "Chemical Compositions, Antifungal and Antioxidant Activities of Essential Oil and Various Extracts of Melodorum

- Fruticosum L. Flowers." *Food and Chemical Toxicology* 48 (10):2754–58.
<https://doi.org/https://doi.org/10.1016/j.fct.2010.07.002>.
- Rodrigues, Vítor H., Marcelo M.R. de Melo, Inês Portugal, and Carlos M. Silva. 2018. "Extraction of Eucalyptus Leaves Using Solvents of Distinct Polarity. Cluster Analysis and Extracts Characterization." *The Journal of Supercritical Fluids* 135
<https://doi.org/10.1016/j.supflu.2018.01.010>.
- Santana, Ana, Catarina Sequeira, Maria Silva, Nanditi Bachu, and Rui Ferreira. 2021. "Portuguese Wine Sme Competitiveness." Nova School of Business and Economics.
- Scopus. 2026. *Scopus*.
- Song, Aihua, Ying Wang, and Yanmei Liu. 2009. "Study on the Chemical Constituents of the Essential Oil of the Leaves of Eucalyptus Globulus Labill from China." *Asian Journal of Traditional Medicines* 4
- Stockley, C, A Paschke-Kratzin, PL Teissedre, P Restani, NG Tejedor, and C Quini. 2021. "Oiv Collective Expertise Document So2 and Wine: A Review." OIV - International Organisation of Vine and Wine.
- Surbhi, Ashwani Kumar, Sarabjit Singh *et al.* 2021. "Eucalyptus: Phytochemical Composition, Extraction Methods and Food and Medicinal Applications." *Advances in Traditional Medicine* 2021 23:2 23 (2)<https://doi.org/10.1007/s13596-021-00582-7>.
- Vally, H., and N. L. Misso. 2012. "Adverse Reactions to the Sulphite Additives." *Gastroenterol Hepatol Bed Bench* 5 (1):16–23.
- Vecchio, Riccardo, Eva Parga-Dans, Pablo Alonso González *et al.* 2021. "Why Consumers Drink Natural Wine? Consumer Perception and Information About Natural Wine." *Agricultural and Food Economics* 2021 9:1 9 (1)<https://doi.org/10.1186/s40100-021-00197-1>.
- Vernhet, Aude. 2019. "Red Wine Clarification and Stabilization." In *Red Wine Technology*.
<https://doi.org/10.1016/B978-0-12-814399-5.00016-5>.
- Xiao, Fan, Tao Xu, Baiyi Lu, and Ruihai Liu. 2020. "Guidelines for Antioxidant Assays for Food Components." *Food Frontiers* 1 (1)<https://doi.org/10.1002/fft2.10>.

Acknowledgments

This work was developed within a framework of awareness for the valorization of natural residues for the manufacture of added-value products under the scope of the Chemical Product Engineering course of the Master in Chemical Engineering at the Faculty of Engineering of the University of Porto. Professor Cláudia Gomes and Doctors Ricardo Santos and Yaidelin Manrique, supervisors of this work, are members of LSRE-LCM – Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, supported by Fundação para a Ciência e a Tecnologia, I.P./MECI through national funds: LSRE-LCM, UID/50020/2025 (<https://doi.org/10.54499/UID/50020/2025>); ALiCE, LA/P/0045/2020 (<https://doi.org/10.54499/LA/P/0045/2020>); and LEPABE, UID/00511/2025 (<https://doi.org/10.54499/UID/00511/2025>) e UID/PRR/00511/2025 (<https://doi.org/10.54499/UID/PRR/00511/2025>); and by Project Queijo Nutri+: Adding nutritional value and longevity to cheese through natural-based ingredients”, nr. 18469 and operation code at the Funds Platform (Balcão dos Fundos) COMPETE2030-FEDER-01485100, co-financed by COMPETE 2030, by Portugal 2030 and by the European Union.