Scientia cum Industria

Special Issue
7º CONGRESSO INTERNACIONAL DE TECNOLOGIAS PARA O MEIO AMBIENTE

PLATINUM OPEN ACCESS
eISSN 2318-5279

Original Research

Recovery of antioxidant compounds from wine lees using membrane technologies

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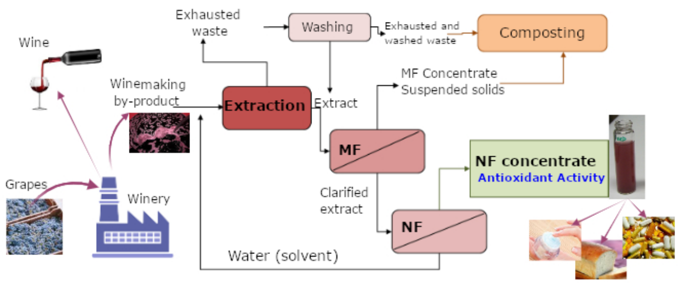

Received: October 31, 2023
Revised: December 2, 2024
Accepted: December 2, 2024
Published: January 30, 2025

Abstract: Considering the substantial volume available and the high concentration of antioxidant compounds in wine lees composition, the present work investigated the use of this winemaking by-product as a raw material for the recovery of anthocyanins and other phenolic compounds. This study explores a process that includes an aqueous extraction step of the antioxidant substances from wine lees, followed by microfiltration to remove suspended material, and a nanofiltration step, resulting in a concentrate rich in anthocyanins and other phenolic compounds. The final product consists of an extract concentrated in natural antioxidant compounds with high antioxidant activity and presenting potential to be used in formulations of pharmaceutical, cosmetic and food industries. The process presented is grounded on sustainability, using eco-friendly solvents and clean technologies, aligning with the principles of circular economy and adding value to wine industry by-products.

Keywords: Anthocyanins, circular economy, membranes, nanofiltration, phenolic compounds, wine by-products.

Introduction

Synthetic antioxidants are widely used in industrial applications, especially in the pharmaceutical, food, and cosmetic industries. However, some of these compounds, such as butyl hydroxyanisole (BHA), butylhydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ), have raised concerns about their safe use, due to their potential toxicological and carcinogenic effects [1]. This factor, associated with the tendency of the growing consumer preference for natural, additive-free, instead of synthetic ones [2], has driven interest and demand for natural antioxidants, as it is assumed that they are perceived as safer and are believed to provide a more effective approach to neutralizing free radicals and reactive species, reducing oxidative stress and associated damage [3].

From this perspective, agro-industrial residues represent a promising alternative for supplying this expanding market, as they consist of abundant and low-cost sources rich in bioactive compounds, mainly phenolic compounds, which are known for their antioxidant properties [4]. According to studies by Ky et al. [5], phenolic compounds act to prevent the oxidation of nucleic acids, lipids, and proteins, preventing degenerative diseases such as cancer, dermal disorders, aging and heart disease. Furthermore, phenolic compounds have also received attention for having notable properties in food preservation [6] and also for acting in protection against brain dysfunction [7].

These factors have stimulated the development of research on the recovery of phenolic compounds and other antioxidant substances from a series of residues and agro-industrial by-products such as apple pomace [8], sour cherry pomace [9], hazelnut shells [10], residues from sugarcane processing [11], dregs from the production of fermented pomegranate drink [12], by-products from citrus processing [13], wood extracts [14], among others. Nevertheless, most studies are related to wastewater from olive oil production [15,16] and by-products from the wine industry, particularly grape pomace [17–19] and grape seeds [20,21]. In fact, grape pomace is the most abundant winemaking by-product, representing around 62%

of total waste; however, lees are also generated in large quantities, representing around 14–25% of the by-products generated in wine production [22].

Despite its significance in terms of the volume of by-products generated, and the fact that the wine industry is responsible for substantial production volumes – in the last decade (2013–2022) the average annual global production reached the level of 270 million hectoliters of wine, from which 3.6 million hectoliters were produced in Brazil [23] – wine lees are still underutilized when it comes to recovering phenolic compounds and other antioxidant substances [24]. It is important to note that lees basically consist of residues that settle in tanks and barrels after fermentation stages, during storage or after some authorized procedure, as well as those obtained by filtration or centrifugation of wines [25]. Lees generated after alcoholic fermentation are referred to as first racking lees, while those originating from malolactic fermentation are known as lees from the second racking [24]. In this way, the lees consist essentially of wine that is saturated with substances settling over time, such as microorganisms (mostly yeast) and their residues, tartrates, phenolic compounds, and polysaccharides, among other substances. Therefore, lees are rich in phenolic compounds and other value-added bioactive substances, characterizing themselves as a low-cost raw material for the recovery of antioxidant compounds [7].

Given these considerations, the present study evaluates the possibility of using wine lees as a raw material for the recovery of antioxidant compounds, especially phenolic substances, using membrane technologies as sustainable methods of valuing by-products from the wine industry.

Experimental Section

The present study investigated the recovery of antioxidant compounds from wine lees through aqueous extraction associated with membrane technologies such as microfiltration (MF) and nanofiltration (NF).

The lees used in this study were obtained from the production of red wine from grapes of the Merlot variety, collected after malolactic fermentation (lees from the second racking), in a winery from Vale dos Vinhedos, Rio Grande do Sul, Brazil. The lees were collected and stored at –20 °C until use.

The aqueous extraction was performed using 800 mL of lees diluted with distilled water to a volume of 8 L and kept under stirring for approximately 30 minutes at room temperature.

Microfiltration of the aqueous extract of wine lees was carried out in bench-scale equipment manufactured by PAM-Membranas Seletivas (Brazil), using a hollow fiber membrane module (MF137) with 590 cm^2 of area and pore size of 0.5 µm. The tests were conducted at 25 °C, pressure of 0.5 bar, and feed flow rate of 200 L h^{-1}, in batch mode and in concentration mode, where the permeate is continuously collected and the retentate is recirculated to the feed tank, as detailed in previous works [25].

The MF permeate was processed by nanofiltration on bench-scale equipment, using a flat sheet membrane with a molecular weight cut-off (MWCO) of 400 Da (NF270, FilmTec, USA) and 14.5 cm^2 of area. These tests were carried out at 5 bar pressure, 25 °C, and feed flow rate of 150 L h^{-1}, in batch and full recirculation mode, where the permeate and retentate are recirculated to the feed tank and samples are collected only for measuring the permeate fluxes and carrying out chemical analyses [3].

The permeate flux and rejection of target compounds were calculated using equations (1) and (2).

$$J_P = \frac{m_f - m_i}{t \times A_m} \quad (1)$$

$$R = \frac{C_A - C_P}{C_A} \times 100 \quad (2)$$

J_P is the permeate mass flux (kg h^{-1} m^{-2}), and m_i and m_f represents the masses (kg) of the sample bottle without and with permeate, respectively. A_m is the membrane area (m^2), t is the permeate collection time (h), R is the percentage rejection of the target compound, and C_P and C_A are the concentrations of the target compound in the permeate and feed, respectively.

During the course of the experiments, the concentrations of total phenolic compounds (TPC) and total monomeric anthocyanins (TMA), as well as antioxidant activity (AA), were closely monitored using analysis protocols outlined in the literature [26–28]. Additionally, pH levels were measured using a multiparameter analyzer (JoanLab, China), turbidity were assessed using a turbidimeter (Alfakit, Brazil), and total suspended solids (TSS) were determined through gravimetry, following the methodology reported in [29].

Results and Discussion

The wine lees used in this study had an acidic character (pH = 3.7) and a highly turbid appearance, with turbidity in the order of 7,000 NTU and total suspended solids of 6,000 mg L^{-1}, making their processing by membrane technologies unfeasible without pre-treatment. The aqueous lees extract, in turn, presented a considerably lower load, reaching 700 NTU of turbidity and 600 mg L^{-1} of TSS, allowing its processing by microfiltration.

In the MF stage, whose initial extract volume was 8 L, around 7 L of permeate was obtained, representing a volumetric recovery of 87.5%. Throughout microfiltration, the permeate flux followed the expected behavior, starting at a high level (70 kg h^{-1} m^{-2}) and continuing to decrease as permeate recovery was raised until reaching 23 kg h^{-1} m^{-2} at the end of the experiment (Figure 1). It is imperative to highlight that in parallel with the increase in permeate recovery, the volume of extract in the feed tank decreases, while the concentration of solutes and particles in the feed tank increases, making it more concentrated, this explains the progressive drop in the permeate flux. Regarding the physicochemical characteristics, the MF permeate presented a slightly pink and transparent color (turbidity less than 1 NTU), with concentrations of total phenolic compounds and total monomeric anthocyanins of 26.6 and 4.2 mg L^{-1}, respectively.

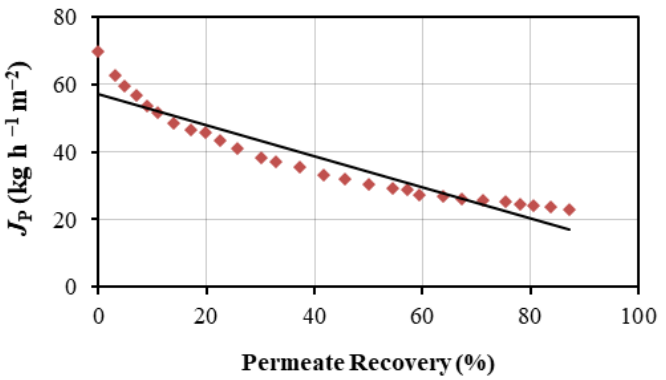

Figure 1. Permeate flux (JP) as a function of permeate recovery in the microfiltration step.

The microfiltration permeate was then subjected to the nanofiltration step with the NF270 membrane, as previously described. In a steady-state conditions and operating at 5 bar pressure, a permeate flux of 36 kg h^{-1} m^{-2} was obtained. Under these conditions, the NF270 membrane presented rejections of 92.6% for total phenolic compounds and total rejection of anthocyanins (100%), which are values similar to previous studies [30]. This behavior is intrinsically linked to the antioxidant activity of the streams evaluated, where the nanofiltration feed (MF permeate) exhibited an antioxidant activity of 10.1 mM Trolox and the NF permeate displayed low antioxidant activity (1.2 mM of Trolox), these results demonstrate that this nanofiltration membrane is capable of concentrating antioxidant compounds, characterized in this study in terms of total phenolic compounds and total monomeric anthocyanins. These results are aligned with data found in the literature [26], where it is reported that this same nanofiltration membrane (NF270) resulted in high rejections of phenolic compounds and permeate samples with low antioxidant activity.

Based on these results, Figure 2 presents an integrated process based on extraction and membrane technologies for recovering antioxidant compounds from wine by-products, specifically wine lees. The first step involves subjecting the by-products or residues to an extraction process. While conventional organic solvents can be used, it is also possible to conduct this process in an eco-friendly manner by employing "green solvents" like water or ethanol-water solutions. Furthermore, extraction can be assisted by other methods such as ultrasound, microwaves or enzymes. Subsequently, MF is used to remove suspended solids from the extract. Finally, NF is applied to concentrate anthocyanins and other phenolic compounds, obtaining a concentrate rich in antioxidant substances. Thus, four streams result from this conceptual process: 1) Exhausted solid waste, which can be subject to composting or other applications; 2) Microfiltration concentrate, which can be used together with exhausted solid waste; 3) Nanofiltration concentrate, enriched in anthocyanins and other phenolic compounds with antioxidant activity, which can be used in pharmaceutical, cosmetic and food industries; 4) Nanofiltration permeate, consisting of the solvent used (water), which can be reused in the extraction stage.

The process illustrated in Figure 2 represents an innovation for the valorization of residues and other by-products from winemaking, as it recovers antioxidant substances such as anthocyanins and other phenolic compounds using a sustainable process, with a lower environmental footprint than conventional processes, avoiding the use of toxic conventional organic solvents. Wine-producing regions such as Serra Gaúcha – the primary wine region in Brazil – have the potential to benefit greatly from implementing a similar process due to the abundance and low cost of raw materials. In 2021, according to the Brazilian Union of Viticulture (UVIBRA) and EMBRAPA/CNPUV, Rio Grande do Sul produced more than 2 million hectoliters of wine [31].

Conclusions

Winemaking by-products and residues contain high levels of phenolic compounds, anthocyanins, and other bioactive substances of great nutritional and biotechnological importance, mainly due to their antioxidant properties. Therefore, these by-products can be used as raw materials for extracting antioxidant compounds, resulting in products with high added value, which have a broad range of applications in formulations in the pharmaceutical, food, and cosmetic industries. It is important to emphasizing that winemaking by-products are generally considered waste from the production process, resulting in costs to the company due to the need for adequate management (treatment and disposal). On the other hand, the recovery of antioxidant compounds can provide gains in economic and environmental terms, allowing an increase in revenue from the commercialization of value-added products (extract with antioxidant capacity), and reducing, at the same time, treatment and disposal costs, what minimize environmental impacts, also promotes the principles of the circular economy. Furthermore, the presented process is based on environmentally suitable solvents and clean technologies like membrane technologies, contributing to sustainable development.

Sci. cum Ind. **2025**, 14(2), e251422. DOI: 10.18226/23185279.e251422

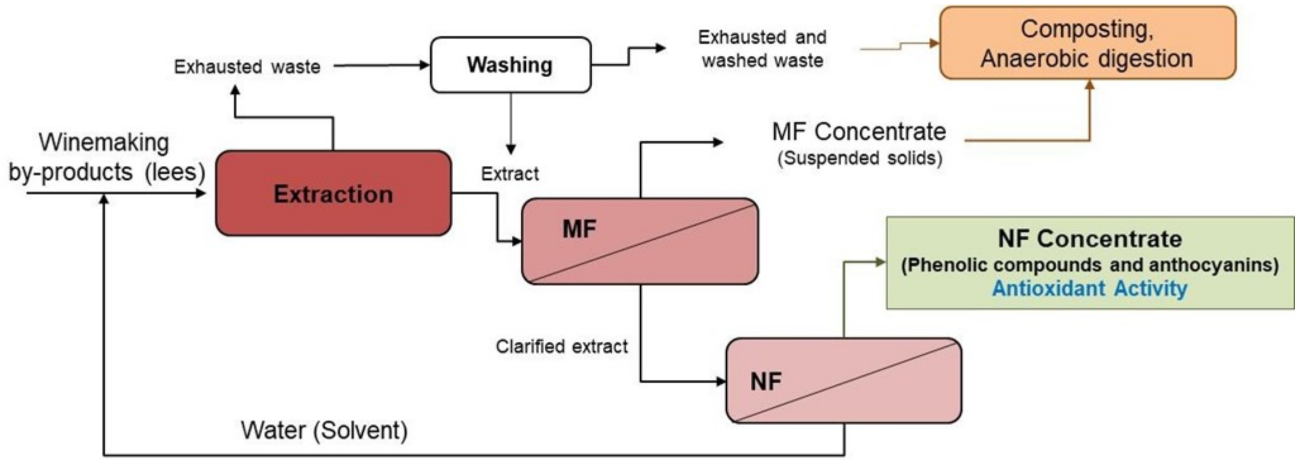

Figure 2. Integrated process based on membrane technologies for recovering antioxidant compounds (anthocyanins and other phenolic compounds) from red wine lees.

Acknowledgments

The authors acknowledge the Brazilian funding agencies (SEBRAE/RS, FAPERGS, CAPES and CNPq), the Portuguese funding agency (FCT) and to Programa Iberoamericano de Ciencia y Tecnología para el Desarollo (CYTED [grant number 323RT0143]) for the financial support, as well as to the Vinícola Almaúnica (Brazil) for providing the wine lees in addition to the financial support.

Authors Contribution

A. Giacobbo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Funding acquisition; A. M. Bernardes: Conceptualization, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition; M. N. de Pinho: Conceptualization, Methodology, Resources, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Conflicts of 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.

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