

Study of antioxidant and antitumoral activity of cork processing wastewaters components fractionated by ultrafiltration membranes

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ABSTRACT

Cork processing wastewater present a severe environmental problem due to its high content in organic matter, such as sugars and non-biodegradable compounds like polyphenols (PPs), namely tannins.

In this work, the assessment of the biological activity and identification of valuable polyphenolic compounds was envisaged. These compounds were obtained using membrane technology, leading to the valorisation of the wastewater components and simultaneously leading to the decrease of its pollutant content.

EXPERIMENTAL

Cork Processing Wastewater

The wastewater samples were collected at a Portuguese cork-processing mill in the region of Montijo, Portugal (38° 42'24.3" N; 8° 58'25.97" W) and were kept refrigerated until the experimental runs were carried out.

Membranes preparation

Two laboratory-made cellulose acetate membranes CA₁ and CA₂ were prepared according to the phase inversion method [1]. The membranes were prepared using casting solutions that contain Cellulose Acetate (polymer), Acetone (solvent) and Formamide (as pore promoter), the two casting solutions compositions and conditions are presented in Table 1.

Table 1 - Casting Solutions composition and casting conditions for UF membranes CA₁ and CA₂ prepared according to the phase inversion method.

Solution composition	CA ₁ (%wt)	CA ₂ (%wt)
Cellulose Acetate	17	17
Formamide	22	35
Acetone	61	48
Casting conditions		
Temperature (°C)	20–25	20–25
Evaporation time (min)	0.5	0.5
Gelation medium	Water (0–3°C)	

Permeation Experiments

The permeation experiments were carried out in an ultrafiltration unit, model Rayflow supplied by TechSep, presented in Figure 1, which is constituted by a feed tank (5L), a pump, a flowmeter, a back-pressure valve, a permeation cell (2 membranes with 147.84 cm² and two manometers.

The installation was operated in i) total recirculation mode, where both the permeate and retentate streams were recirculated to the feed tank, ii) concentration mode, where only the retentate stream was recirculated to the feed tank while the permeate stream was continuously drawn, and iii) diafiltration/concentration mode, where an amount of solvent (is this case water) was added to the system equal to the permeate drawn out, Figure 2.



Fig. 1 - Ultrafiltration unit Rayflow

Anti-tumour activities of the wastewater and fractions

The wastewater and the fractions generated in the UF experiments were used to evaluate the antioxidant activity, the cytotoxic and antiproliferative effect.

The antioxidant activity of the wastewater and the fractions was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

MCF7 cells (ATCC® HTB-22, human mammary gland adenocarcinoma epithelial cell line) were used for cytotoxicity studies using the MTT viability test, according to Mosmann method [3].

In order to evaluate the cytostatic potential of wastewater and fractions, MCF7 cells were cultured with increasing concentrations of samples added to the cell culture medium at seeding. After incubation for 72 h, the samples solutions were replaced by 0.05 mg/mL MTT solution and the assay of quantification was performed as above described. Cell viability was expressed as a percentage of live treated cells considering 100% viability of control cells incubated with culture medium.

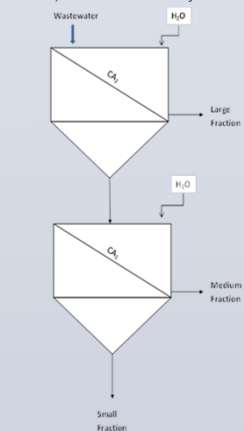


Fig. 2 - Permeation experiments scheme in concentration mode and in diafiltration mode using CA₁ and CA₂ membranes to obtain several fractions from cork wastewater.

RESULTS AND DISCUSSION

Ultrafiltration membranes were characterized in terms of hydraulic permeability (Lp), rejection coefficients to salts and Molecular Weight Cut-Off (MWCO), Table 2.

	CA ₁	CA ₂
Lp (Kg/h/m ² /bar)	2.424	71.582
f NaCl (%)	12	0.5
f Na ₂ SO ₄ (%)	48.8	3.8
MWCO (Da)	2979	74111

Table 2 - Membrane characterization. UF operation conditions, Feed flow rate: 180 L/h, Temp. : 25°C, Pressure: 1-3 atm.

Characterization of cork boiling wastewater and fractions

The three wastewater fractions (small, medium and large) were characterized in terms of Total polyphenols, Tannins and polysaccharides, Table 3.

Parameter	Wastewater (W)	Small Fraction (S)	Medium Fraction (M)	Large Fraction (L)
Total Phenols (g GAE g ⁻¹)	0.100 ± 0.005	0.053 ± 0.004	0.079 ± 0.004	0.156 ± 0.005
Tannins (g TAE g ⁻¹)	0.062 ± 0.004	0.025 ± 0.002	0.031 ± 0.001	0.074 ± 0.003
Total polysaccharides (g glucose g ⁻¹)	0.217 ± 0.026	0.128 ± 0.04	0.065 ± 0.008	0.278 ± 0.052
TOC (ppm)	2260	353	428	1602

Table 3 - Characterization of cork boiling wastewater and fractions: Total phenols, Tannins, Total polysaccharides and TOC. The fractions were obtained according to the procedure described in the Materials and Methods section-Wastewater permeation experiments. GAE and TAE refers to gallic acid equivalents and tannic acid equivalents, respectively.

Table 4 - Quantification of Quinic, Gallic, Protocatechuic and Ellagic acids in the different fractions small (S), medium (M) and large (L) recovered and in the wastewater (W) at a concentration of 1 mg / mL.

Component (µg/mg)	W	S	M	L
Quinic Acid	269	267	161	38
Gallic Acid	9	20	4	-----
Protocatechuic Acid	2.61	3.55	1.61	0.89
Ellagic Acid	6	-----	-----	9

Antioxidant activity of cork boiling wastewater and fractions

	EC ₅₀ (µg/mL)
Wastewater	23.48 ± 1.38
Small Fraction	38.87 ± 3.59
Medium Fraction	35.68 ± 2.84
Large Fraction	23.79 ± 1.25
Quinic Acid*	1.5 mg/mL (5% AA)
Gallic Acid	0.54 ± 0.02
Ellagic Acid	1.84 ± 0.07

Table 5 - Determination of EC₅₀ concentration achieving 50% of Antioxidant Activity (AA) from cork wastewater, from fractions recovered and from commercial standards.* EC₅₀ not determined for quinic acid

The wastewater and the fractions were tested for antioxidant activity and for the capacity to inhibit the proliferation of the growth of human breast carcinoma cell lines, MCF-7. The fractions revealed high antioxidant activity ranging from 23.48 ± 1.38 to 38.87 ± 3.59 µg/mL. The fractions demonstrated not to be toxic against carcinoma breast cancer cell lines MCF-7, but efficient as cytostatic, i.e. inhibitors of cell proliferation, with values of IC₅₀ ranging from 0.20 ± 0.003 to 0.46 ± 0.02 mg/mL [2].

CONCLUSIONS

- The cork wastewater revealed to be a source of bioactive compounds. Additionally, the use of membrane technology to obtain various fractions was an efficient strategy to reduce the wastewater organic load and to recover high value phenolic compounds in three fractions such as Small, Medium and Large fraction. This type of approach is a cost-effective and environment-friendly process.
- The wastewater and isolated fractions, mainly the Large fraction, revealed excellent antioxidant activity and also, a novel ability was demonstrated such as the potential to inhibit cancer cells proliferation. Therefore, cork processing wastewater may constitute a source of value-added marketable products.

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