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## Introduction

Grape by-products, such as skins and seeds generated during winemaking, represent a valuable source of bioactive compounds and dietary fiber. These residues are rich in phenolics—particularly anthocyanins—with recognized antioxidant, antimicrobial, and anti-inflammatory activities. Their valorization aligns with circular economy principles, promoting waste reduction and the development of sustainable ingredients.

The exploitation of these materials for food, nutraceutical, and packaging applications provides an opportunity to replace synthetic additives with natural alternatives.

The main objective of this work was to characterize and valorize grape by-products as natural sources of bioactive compounds and antioxidants for sustainable industrial applications.

The **NOVAPACK Project** aims to integrate grape by-products into sustainable value chains through their physicochemical characterization and the extraction of bioactive compounds (Figure 1).

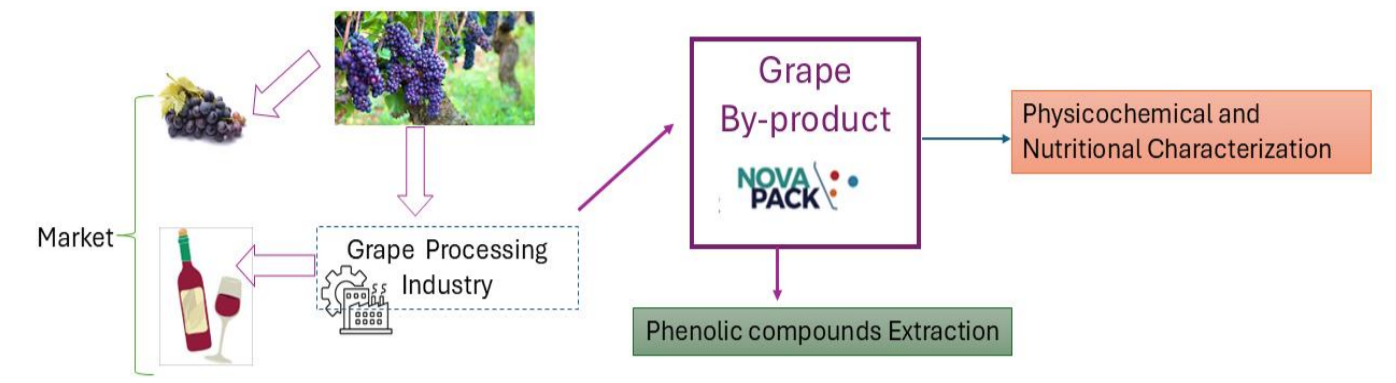


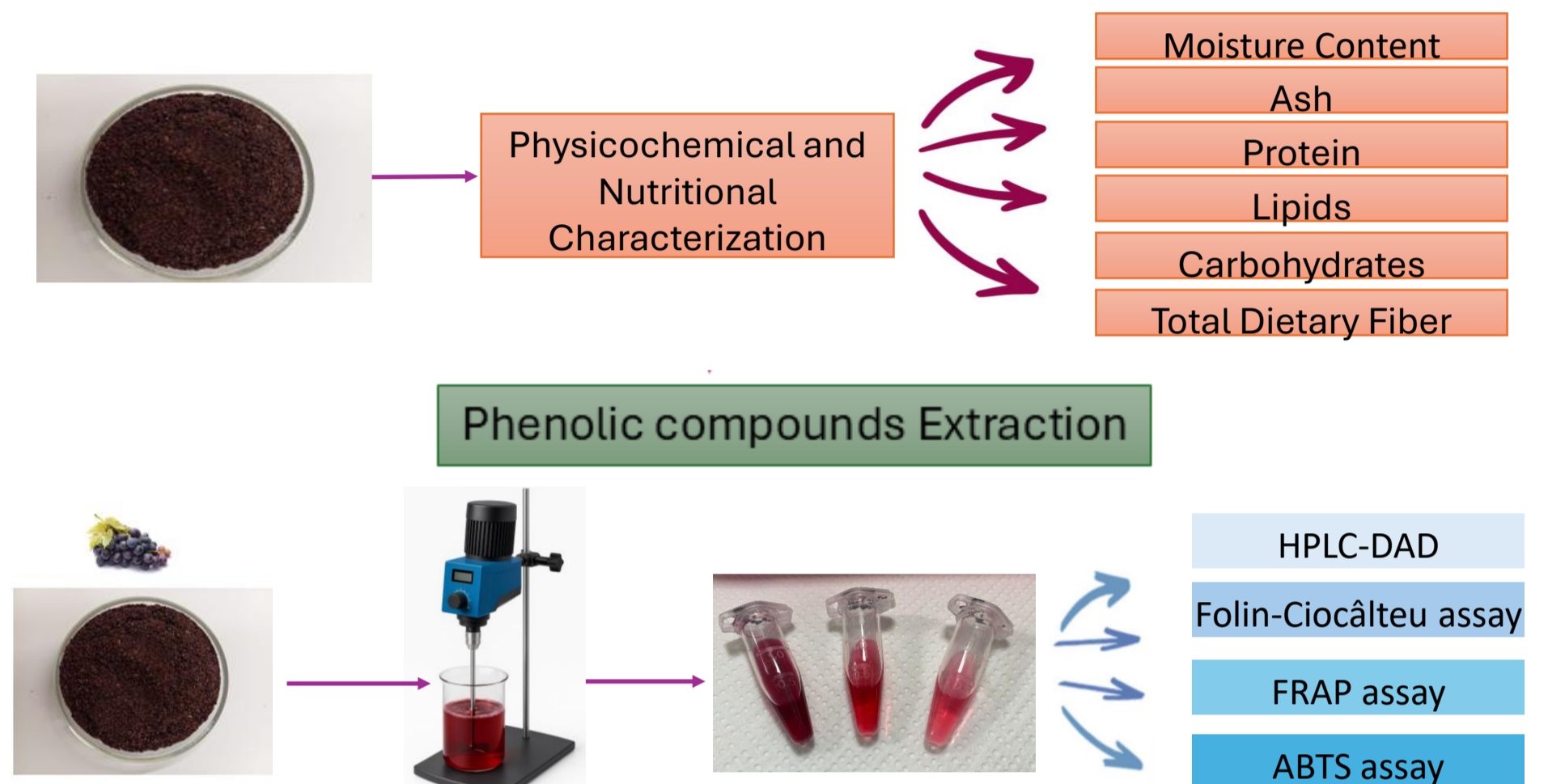
Figure 1. Integrated Valorization Flow of Grape By-Products in the NOVAPACK Project

## Experimental

Table 1- Origin and Type of Grape By-Products used in the project.

Origin	Supplier	Type of material
Grape Portugal	Sparkling wine production company (Casa Senhorial do Reguengo)	Seeds, pomace, membranes, and skins

- Solvents (50:50, v/v):
- 1% citric acid (CA)/ethanol
- 1% lactic acid (LA)/ethanol
- Aqueous ethanol
- Aqueous CA
- Grape concentrations: 1%, 5%, and 10% (w/v)



## Results

Table 2 – Proximate Physicochemical Characterization of Grape by-products.

Components	Grape by-product
	Batch: March 2025
Moisture (% w/w)	3.9 ± 0.0
Ash	5.07 ± 0.1
Protein	10.1 ± 0.1
Lipids	11.9 ± 0.1
Carbohydrates	69.2 ± 0.3
Total Dietary Fiber	68.30 ± 2.8
Insoluble Dietary Fiber (%)	64.48 ± 2.4
Soluble Dietary Fiber(%)	3.82 ± 0.2

Results are expressed as mean values ± SD from three replicates

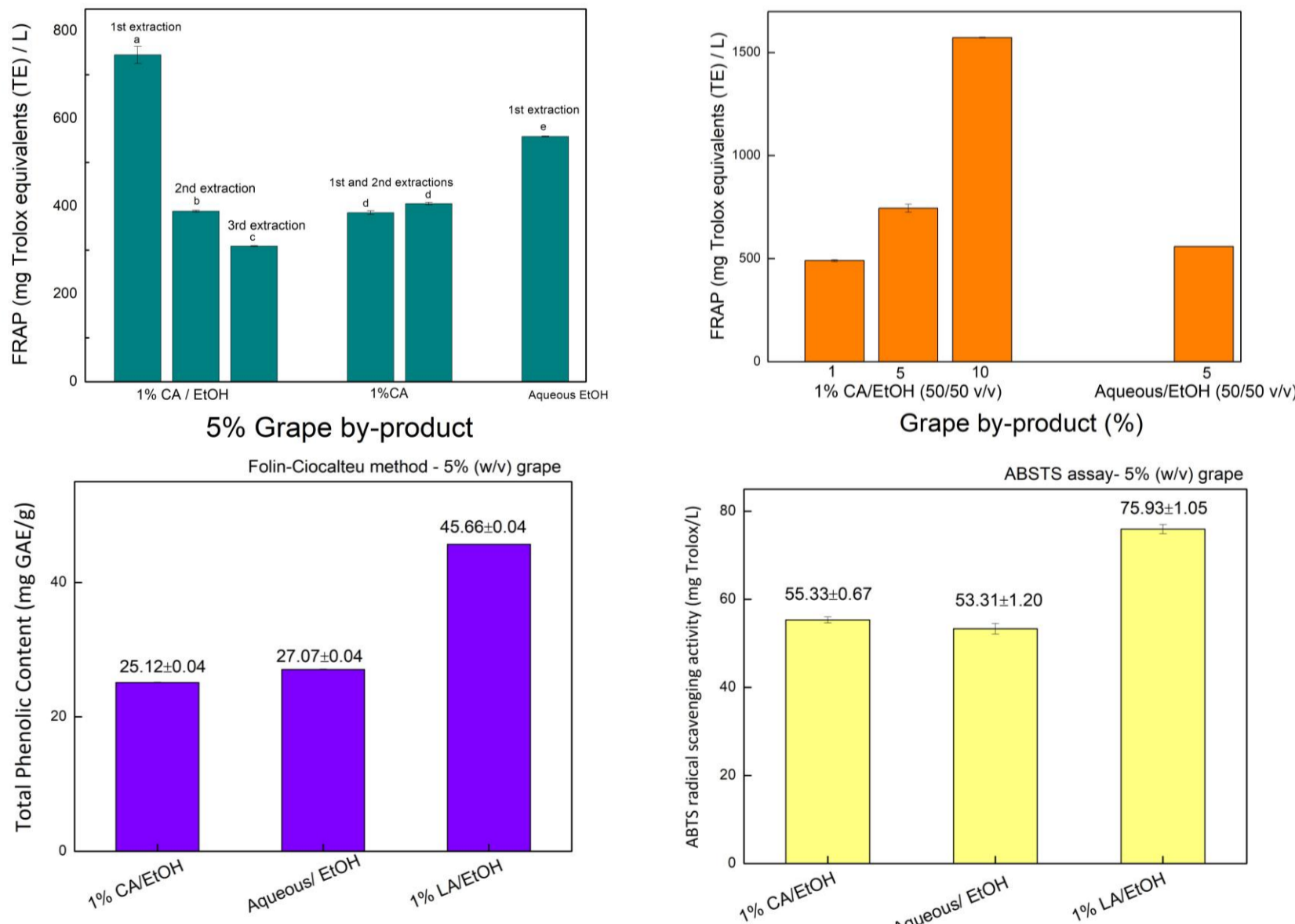


Figure 4- Antioxidant and Phenolic Profile of Grape By-products

Table 3 - Effect of extraction on anthocyanin recovery from grape by-product.

Amostra	Delphinidin-3-O-glucoside (mg/g)	Petunidin-3-O-glucoside (mg/g)	Malvidin-3-O-glucoside (mg/g)
1% grape	4.41 ± 0.22	2.95 ± 0.15	13.54 ± 0.88
5% grape	3.29 ± 0.16	2.30 ± 0.12	10.96 ± 0.55
10% grape	2.96 ± 0.15	2.15 ± 0.11	10.02 ± 0.50

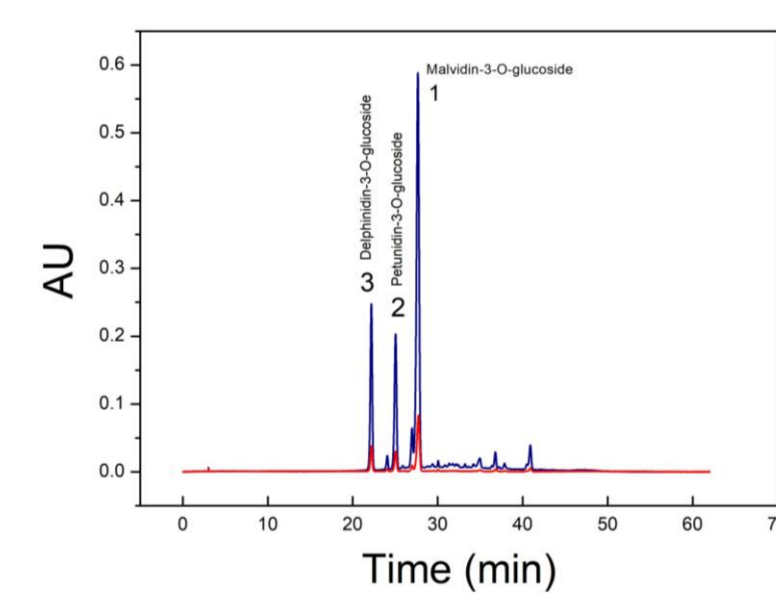


Figure 2- HPLC-DAD chromatograms (520nm) of grape extracts at different concentrations in 1% CA/ethanol (50:50 v/v).

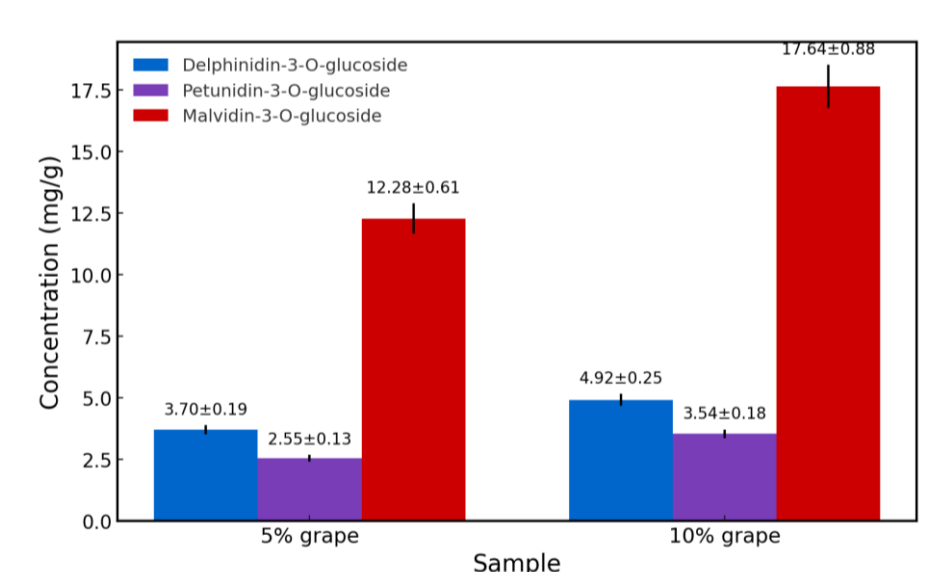


Figure 3- Anthocyanin concentration in grape extracts (1% LA/ethanol)

Table 4 - Anthocyanin concentration (mg/g) in sequential extractions using Turrax (5% grape – 1% CA/ethanol).

Extraction	Delphinidin-3-O-glucoside (mg/g)	Petunidin-3-O-glucoside (mg/g)	Malvidin-3-O-glucoside (mg/g)
1st extraction	3.29 ± 0.16	2.30 ± 0.12	10.96 ± 0.55
2nd extraction	0.82 ± 0.04	0.58 ± 0.03	2.73 ± 0.14
3rd extraction	0.41 ± 0.02	0.29 ± 0.01	1.15 ± 0.06

## Discussion

- ✓ The grape by-product is a rich source of insoluble dietary fiber and bioactive compounds, highlighting its potential as a sustainable ingredient for functional food applications (Table 2)
- ✓ The HPLC-DAD analysis revealed Delphinidin-3-O-glucoside, Petunidin-3-O-glucoside, and Malvidin-3-O-glucoside as the major pigments, with Malvidin being predominant, confirming the efficiency of the extraction process (Figure 2)
- ✓ Anthocyanins were mainly recovered in the first extraction, with a marked decrease in subsequent ones. Malvidin remained predominant, confirming its stability and high solubility under the extraction conditions (Table 4).

- ✓ The extraction system using **1% lactic acid/ethanol** demonstrated higher efficiency than **1% citric acid/ethanol**, especially for **Malvidin-3-O-glucoside**, which remained the predominant anthocyanin (Figure 3 and Table 3).

- ✓ The 1% LA/EtOH extract presented the highest phenolic content (≈45.7 mg GAE/g) and antioxidant activity in both ABTS (≈75.9 mg Trolox/L) and FRAP assays.

Extracts obtained with 1% CA/EtOH and aqueous/EtOH showed moderate results (≈25–27 mg GAE/g; 53–55 mg Trolox/L).

The first extraction step yielded the greatest reducing capacity, while subsequent extractions showed lower activity. (Figure 4).

## Conclusion

- Grape by-products showed rich physicochemical and nutritional properties, with high fiber, carbohydrate, and protein contents.
- 1% LA/EtOH extraction achieved the highest phenolic and antioxidant yields, confirming its eco-efficient potential.
- Malvidin-3-O-glucoside was the predominant and most stable anthocyanin across all extraction systems.