

APPLICATION OF ADSORPTION RESIN TECHNOLOGY AND FCPC CHROMATOGRAPHY FOR THE RECOVERY OF POLYPHENOLS AND STILBENOIDS FROM GRAPE POMACE



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Grape pomace is an agricultural waste produced in huge amounts during the wine making procedure. In Greece the grape production is about 100.000 tn while 400.000.000 lt of wine are produced annually in about 400 wineries. Grape pomace consists the 17% of the grape biomass that is used for wine production. It is a very rich source of bioactive compounds and especially stilbenoids, phenolic acids and flavonoids. Under the framework of the Life-Environment Programme "DIONYSOS" we designed and developed at pilot scale a system for the treatment of grape pomace with the aim to recover the contained stilbenoids/polyphenols and reduce the environmental problems. The treatment system consists of the following four main successive individual sections:

(A) EXTRACTION-FILTRATION

The first section of the treatment system includes the collection, air-drying, pulverization and extraction of grape pomace. The dry grape pomace powder (100 Kg) is extracted twice with EtOH (250 lt) with mechanical stirring for 12 h. Then the extract is separated from the powder and diluted with ten volumes of water. The resulting water-alcohol is finally filtered using a bagfilter with 25µm porosity.

(B) SELECTIVE ADSORPTION OF POLYPHENOLS

The second section of the treatment procedure includes the pass of the filtered water-alcohol extract through a series of specialised adsorbent resins in order to achieve the decolourisation and the removal/ recovery of the polyphenols content.

1st stage. This stage aims at the capture of the polyphenols content.

Filtered extract (500 lt) is fed into a column of 160 cm height and 40 cm diameter, which is filled in with 100 lt of XAD4 resin. The extract is fed into the resin through a PVC pipe using a pump. The feeding flow rate is 125 lt/h. The resin total feeding duration is 4 h.

Afterwards in order to regenerate/ reactivate the resin, the next regeneration/ reactivation procedure is followed:

- Resin washing by counter-current water flow under 3 bar pressure for 15 minutes, in order to remove the suspended solids which were settled on the resin's surface.
- Rearranging of the resin's medium inside the column by co-current water flow for approximately 5 minutes.
- Resin regenerating by co-current feeding of ethanol for 35 minutes, with flow rate 250 lt/h.
- Resin washing by co-current water flow for approximately 30 minutes.

After the completion of the above procedure, the resin is ready to be used again
2nd stage. This stage aims at the capture of the organic substances, which produce the red colour of the extract. The effluent from the first resin is re-fed, under the same operation conditions of the 1st stage, into a second column (filled in with 100 lt of XAD7HP resin). After the completion of the 2nd stage, in order to regenerate/ reactivate the resin, the regeneration/ reactivation procedure of the 1st stage is followed.

The water-alcohol grape pomace waste after its pass from the previous stages is an odourless, light yellow coloured liquid, which does not contain any polyphenolic substances. All contained polyphenols have been successfully adsorbed. The contained alcohol in the liquid waste is recovered by distillation.

(C) THERMAL TREATMENT-SOLVENT RECOVERY

The third section of the treatment procedure aims at the recovery of the organic solvents mixture, which has been used in section B of the system and the obtaining of the dry enriched extracts. Each one of the organic solvents mixtures that has been used respectively in the 1st and 2nd stage of section B, is evaporated separately in a thermal treatment system consisted of Rotary evaporators BÜCHI. The organic solvents mixtures are thermally treated under conditions of 45°C temperature and 100 mbar vacuum. The condensation of the produced organic solvents vapours is performed by using a coolant (glycol) of -7 °C temperature.

(D) PURIFICATION OF RESVERATROL BY FCPC

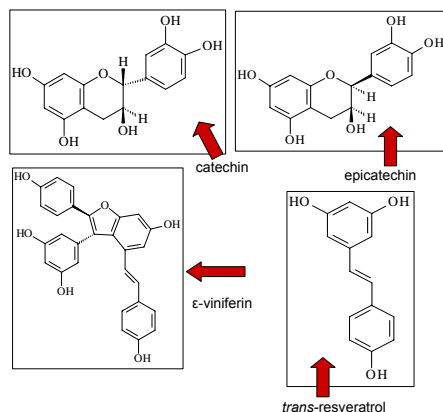
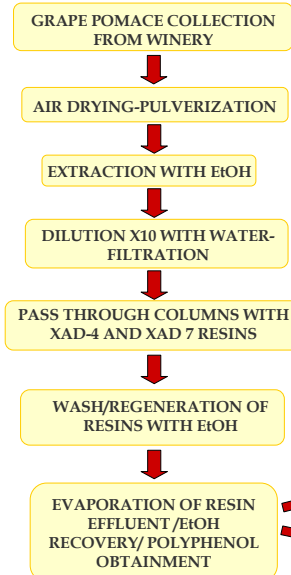
The fourth section aims at the purification of the stilbenoids and other polyphenols contained in the enriched extract coming from section C, using FCPC chromatography.

A part of the residue (18g) of section C (resin XAD-4), was dissolved in H₂O (1L) and extracted with EtOAc (1L). The EtOAc layer was evaporated (5.2g), dissolved in 30 ml of H₂O, and then was submitted to CCC (Counter Current Chromatography), using an FCPC (Fast Centrifuge Partition Chromatography) apparatus. The system of solvents used in this procedure was CHCl₃/MeOH/ H₂O : 10/10/6 (1L). The capacity of the column was 1L, the rapidity of the rotation was 500rpm and the flow was 15ml/min.

At first the mobile phase (CHCl₃) was running in "ascending" mode through the stationary phase (MeOH/H₂O). The mode was changed to "ascending" after 48 fractions (720 ml) and the mixture of MeOH/H₂O became the mobile phase. Totally, 89 fractions were collected. Finally 17 mg of trans-resveratrol were isolated by the above mentioned process along with catechin, epicatechin and viniferin.

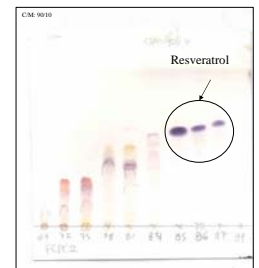
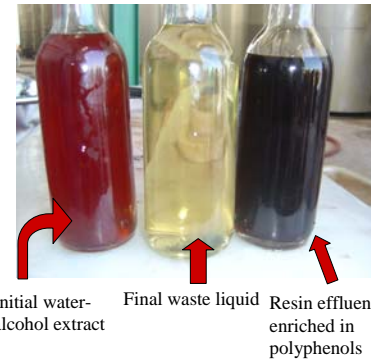
THE FINAL OUTCOME OF THE WHOLE PROCEDURE IS:

- an extract enriched in stilbenoids and polyphenols with high antioxidant activity and high added value
- pure resveratrol (1 gr per 100 Kg of dry grape pomace)
- an odorless yellowish wastewater with a 99.5% reduced content in polyphenols
- an extract containing the coloring substances of the grape skin



ENRICHED EXTRACT FORMULATION

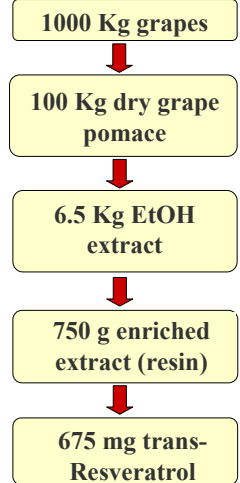
PURIFICATION BY FCPC



ENRICHED GRAPE POMACE EXTRACT FORMULATION

The obtained extract after the resin treatment is a semisolid product that cannot be easily handled. For this purpose the extract is mixed with maltodextrin diluted in water and submitted to spray-drying. The final product is a red fine powder that can be easily consumed after dilution in a glass of water. Based on the quantity of the contained resveratrol in the extract and the corresponding grapes, consumption of approx. 600 mg of the extract is equivalent with the consumption of 1 Kg of grapes.

Resveratrol purification by FCPC



Main compounds of the enriched red grapes extract	mg/g (by HPLC analysis)
Epicatechin	4.32
Catechin	2.72
Galic acid	2.07
trans-resveratrol	0.9
Rutin	0.47
ε-Viniferin	0.42
p-Coumaric acid	0.28
Ferulic acid	0.14
Quercetin	0.04

EXTRACTION ADSORPTION

SOLVENT RECOVERY