Valorization of wine wastes for added-value and/or biological products

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Abstract: Evaluation of wine wastes for isolation of added value products with applications in food and pharmaceutical industries, cosmetology etc was carried out. Samples of different wine wastes from red and white vinification were examined for their content in tartaric acid as well as in total phenolics and sugars. Based on chemical precipitation and separation processes, tartaric acid crystals were recovered at 4.48 and 3.57 g-TA/100g-dry mass of red and white tartar wastes respectively, whereas phenolic compounds extracted from red marc were the highest (25 mg/g) compared to other wine wastes with values of 17 mg/g for white marc, 13 mg/g for white lees, 3 mg/g for white tartar wastes and 15.5 mg/g for red tartar wastes. Red marc sample exhibited the highest (94%) radical scavenging activity as well as significant inhibition of platelet aggregation induced by collagen in vitro experiments. In an attempt to isolate phenolics with high antioxidant activity, we had previous reported on selective adsorption using various sorbents such as aluminum oxide and zeolite. Here, encapsulation with sodium alginate and sodium alginate-PEG mixtures was investigated. It was found that ~89% of the initial phenolics can be entrapped. Release experiments though, extracted ~67% of the initial phenolic compounds possessing the ~56% of the initial radical scavenging activity, supporting the need for continuation of optimization of release studies.

Key-Words: wine wastes, tartaric acid, encapsulation, platelet aggregation, phenolics.

1 Introduction

Grapes are one of the world’s largest important fruits, their annual production is nearly 60,000,000 tn and mostly (80%) is used in winemaking industry [1]. Enological industries release a large amount of wastes, comprising of 50% skins, 25% seeds and 25% stalks. These contain a large amount of valuable secondary by-products such as calcium tartrate, phenolics, sugars etc., whereas their free disposal, increase the chemical and biochemical oxygen demand.

Tartaric acid is a dihydroxy dicarboxylic acid which is one of the main acids found in grapes [2]. It lowers the quality of wine due to the precipitation. Precipitation of calcium tartrate and potassium hydrogen tartrate is of major concern in the bottled wines. To avoid crystalline deposits, wines are stabilized in the cellar first and the precipitated salts are removed by filtration. These tartar wine wastes can be processed for recovery of tartaric acid, a valuable by-product, which can be subsequently used in food, chemicals, pharmaceutical, cosmetic, building and metallurgical industries [3,4].

Phenolic compounds are potent antioxidants, possessing antiplatelet activity, whereas exhibit beneficial effects towards cardiovascular diseases, cancer e.t.c [1,5]. The isolation of such chemical constituents from low-cost industrial wastes using various adsorbents [6] could greatly reduce their production costs and increase the margin profit of the products. In addition, encapsulation is a method that can be used to preserve their stability, bioactivity and bioavailability. Apart from that, capsules can release their contents at controlled rates under specific conditions rendering phenolics use more applicable [7].

2 Problem Formulation

Enological wastes are mainly used as animal feed, fertilizers and/or alternatively can be incinerated or buried. However due to their high content in antioxidant compounds and calcium tartrate, their isolation and subsequent valorization of wine wastes is more than necessary. The objectives of our study were: (1) to recover the tartaric acid from red and white tartar wastes and (2) to separate
phenolic compounds from its natural mixtures and encapsulate them for certain purposes.

2.1 Wine wastes
Grape marc, lees and tartar wastes (malagouzia and syrah variety) were kindly provided by Ktima Gerovassiliou, a wine-making factory in Epanomi (Thessaloniki, Greece) in the vintage 2013. Moisture content was determined by drying a pre-weighted amount of the wastes in an oven (Heraeus KT 5050, West Midlands, U. K.) at 70 °C for 10 h.

Wine wastes samples (1 g dry weight) were extracted with a certain volume of ethanol in a sonicator (35°C, 20 min.) to increase the yield of extracted phenols and their antioxidant activity.

2.2 Recovery of tartaric acid
The red and white tartar waste (100 g dry weight) were treated with 20 ml of concentrated HCl, under continuous stirring for 1 h at 70 °C, and subsequently centrifuged at 2000 g (Kubota 5922, Tokyo, Japan), followed by vacuum filtration. The extracted tartaric acid was precipitated as calcium tartrate by the subsequent addition of CaCO₃ and CaCl₂·2H₂O (1.623 and 1.326 g for red and white tartar waste respectively). The precipitated calcium tartrate was dried and washed with ethanol for the removal of the organic impurities, followed by treatment with dil. H₂SO₄ under boiling for 45 min and then cooling to remove CaSO₄. After that the collected tartaric acid solutions were decolorized by means of activated carbon and finally, crystallized on a rotary evaporator at 70 °C. The tartaric acid crystals were dried in vacuum desiccator containing silica gel beads followed by qualitative and quantitative analysis. The average tartaric acid content present in both tartar wastes was the mean value of three experiments at the same conditions.

2.3 FTIR and Ion chromatography analysis
Potassium bromide disks were prepared by mixing 1 mg of lyophilized tartaric acid crystals with 200 mg KBr, and the spectra were recorded from 400 to 4000 cm⁻¹ with a resolution number 2 cm⁻¹ using FTIR Spectrophotometer (Equinox 55, AXS Bruker, USA). Tartaric acid was analyzed by ion chromatography ( Dionex series 4500i, USA) using column (PRD × 300, 25 × 4.1 mm). The mobile phase was 0.001 N H₂SO₄ at a flow rate of 0.8 ml/min and the detection wavelength was set at 210 nm.

2.4 Platelet aggregation
Platelet aggregation experiments were performed by a conventional photometric technique in a four channel aggregometer, at 37 °C, with continuous recording of light transmission, according to the method of Born [8]. Collagen was used as aggregation agent. Platelets were obtained from venous blood of healthy donors. The blood was immediately mixed with 3.8% sodium citrate solution and was centrifuged at 1000 rpm for 10 min to yield platelet rich plasma (PRP). The aggregation was determined using the aggregometer by recording the increase of light transmission. The aggregometer calibration was performed using platelet poor plasma (PPP; 100% T) obtained by centrifugation (1500 × g, 15 min) [9].

2.5 Encapsulation
The encapsulating agents were 3% sodium alginate solution with 1% PEG (polyethylene glycol). The white marc extract was mixed with the sodium alginate solution and added drop wise into a calcium chloride solution (1.5%). The beads were maintained to harden for 30 min and then filtered through [10].

The beads obtained were suspended in ethanol at room temperature and kept for 72 h. To analyze the release effect of total polyphenol content Folin-Ciocalteau method [11] and antioxidant activity was measured using DPPH assay [12].

The encapsulation efficiency was calculated according to the equation below:

\[ EE \% = (APC/TRP) \times 100 \]  

Where: APC is the actual phenolic content and TPC is the theoretical phenolic content [13].

3 Problem Solution

3.1 Wine wastes
The following table gives an indicative annual production of wine wastes from Gerovassiliou industry.
### Table 1. Indicative annual production of wine waste.

<table>
<thead>
<tr>
<th>White Vinification</th>
<th>tn/yr</th>
<th>Red Vinification</th>
<th>tn/yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grapes</td>
<td>400</td>
<td>Grapes</td>
<td>100</td>
</tr>
<tr>
<td>Marc</td>
<td>120</td>
<td>Marc</td>
<td>25</td>
</tr>
<tr>
<td>Lees</td>
<td>32</td>
<td>Tartar wastes</td>
<td>2-3</td>
</tr>
<tr>
<td>Tartar wastes</td>
<td>2-5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2 Qualitative analysis of tartaric acid

Fig 1 (a and b) shows the FTIR spectra of tartaric acid from red and white tartar wastes respectively. The figures indicated the characteristic bands of carboxylic groups at 1388, 1383 and 1389 cm\(^{-1}\) for the standard compound, and the tartaric acid recovered from red and white wastes. According to Lambert et al. [14], the observed peak positions may be attributed to carboxylic groups present on the samples.

![FTIR spectra](attachment:ftir_spectra.png)

**Fig. 1** FTIR spectra of (a) standard tartaric acid and (b) recovered from red tartar wastes

Tartaric acid purity and identification was further elucidated by ion chromatography analysis.

3.2 Quantitative determination of tartaric acid

Calcium tartrate was precipitated with CaCO\(_3\) and CaCl\(_2\) at 5.17 and 4.02 g-TA/100 g-dry mass for red and white tartar wastes respectively. The tartaric acid crystals were recovered at 4.48 and 3.57 g-TA/100 g-dry mass of red and white tartar wastes respectively. The results further showed that tartaric acid contents with corresponding calcium tartrates were around 86.65 and 88.80% in red and white tartar wastes respectively. According to Amerine et al. [15], most of the tartar cream separates during the fermentation of red must on the skins, whereas white grapes were nearly always pressed before fermentation and there is no fermentation on the skins, resulting the tartrate is richer in red tartar waste than white one. It was also reported that pomace from dry red wine making was richer in tartrates than pomace from dry white wine making [16].

3.3 Platelet aggregation

Table 2 gives the content in phenolics and % DPPH inhibition of wastes from Gerovassiliou industry.
Table 2. Wine wastes content in total phenolics and antioxidant activity.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total Phenolics (mg/g)</th>
<th>% Inhibition of DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marc</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>17.00±1.73</td>
<td>91±1.73</td>
</tr>
<tr>
<td>Red</td>
<td>25.00±2.69</td>
<td>94±2.12</td>
</tr>
<tr>
<td><strong>Lees</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>13.00±2.72</td>
<td>65±2.74</td>
</tr>
<tr>
<td><strong>White Tartar wastes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>3.0±0.53</td>
<td>55±24.34</td>
</tr>
<tr>
<td>Supernatant (mg/ml)</td>
<td>3.60±0.86</td>
<td>n.d.(^{(1)})</td>
</tr>
<tr>
<td><strong>Red Tartar wastes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>6.8±0.72</td>
<td>7.0±0.33</td>
</tr>
<tr>
<td>Residue and Lees</td>
<td>15.50±2.45</td>
<td>6.5±0.38</td>
</tr>
<tr>
<td>Supernatant (mg/ml)</td>
<td>1.5±0.89</td>
<td>22±2.19</td>
</tr>
</tbody>
</table>

* Each value is presented as mean ± SD (n=3)
** All data were statistical significant at \( p < 0.05 \).
\(^{(1)}\) not determined

Red and white marc, due to their high antioxidant activity were also tested for antiplatelet activity in vitro. Fig 3 shows the inhibitory activity of the tested wastes. Both red and white marc showed strong inhibitory activity.

![Fig 3. Effect of collagen (1), red (2) and white (3) marc on human platelet aggregation induced by collagen.](image1.jpg)

3.4 Encapsulation

Encapsulation technique provides stability till the release of the compound is required. Here, the phenolic compounds, from white marc extract, were encapsulated successfully at 89%, whereas the percentage of release was 67% in 24 h (Figure 4). The phenolics released possessed 56% of initial antiradical activity.

![Fig 4. Effect of time on release analysis study of total phenolics from alginate-PEG solution in ethanol. All data were statistical significant at \( p < 0.05 \). All experiments were performed in triplicate.](image2.jpg)

4 Conclusion

Recovery of tartaric acid by chemical precipitation and crystallization was estimated to 44.8 Kg/tn and 35.7 Kg/tn of red and white tartar wastes respectively.

Based on our results, phenolics in crude extracts from red and white marc could be expressed as 25 Kg/tn and 17 Kg/tn of wastes respectively. These values are significant given the biological activity of the products (antioxidant and antiplatelet).

Phenolics were successfully encapsulated at 89% of their initial concentration, whereas the release of them was not so sufficient (67%). Therefore more studies are needed towards this direction.

Considering the annual production of these wastes it seems more profitable for the industry to proceed with valorization than management of the wastes.

Acknowledgements

The research work was supported by "11SYN\_2\_1992" action "COOPERATION 2011" of EYDE-ETAK funded by the Operational Program "Competitiveness and Entrepreneurship" (EPAN-II).

References:

[1] Mildner-Szkudlarz, S., Zawirska-Wojtasiak, R., & Gośliński, M., Phenolic compounds from winemaking waste and its antioxidant activity towards oxidation of rapeseed oil,


