Extraction of polyphenols from grape seeds by unconventional methods and extract concentration through polymeric membrane

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ABSTRACT

The aim of this work was to apply unconventional extraction methods including ultrasonication (US), high voltage electrical discharge (HVED) and pulsed electric field (PEF) to extract polyphenols from grape seeds and concentrate the extracts by ultrafiltration. A comparative evaluation of the three extraction methods for release of polyphenols from grape seeds was investigated. The results show that the three methods, acting through different extraction mechanisms, have a significant effect on the size of the fragments generated and extraction efficiency. By measurements of the quantity of polyphenols released and total energy supplied, extraction efficiency was evaluated. It was shown that with the increase of energy supplied, the amounts of polyphenols released increases for all the three extraction methods. At the same energy consumption, HVED permitted higher polyphenol extraction than the other two methods.

Aqueous extracts from grape seeds were processed using ultrafiltration (50 kDa) membrane to obtain fractions enriched in phenolic compounds. During clarification, extracts obtained by HVED showed the highest specific cake resistance followed by US and PEF. According to Ruth–Carman’s model, the filtration processes were governed by cake formation mechanism. The physico-chemical properties of the permeate and retentate after membrane processing were also evaluated. High retention of polyphenols in the retentate was observed.

Keywords: polyphenol extraction; high voltage electrical discharge (HVED); pulsed electric field (PEF); ultrasonication (US); ultrafiltration

INTRODUCTION

Grape seeds are a by-product obtained after wine or juice making and present a good source of functional compounds such as polyphenols, which are very valuable due to their antioxidant capacity. The methods commonly employed for polyphenols recovery from winery by-products make use of organic solvents like ethanol or acetone [1]. The health concerns have sparked research into the safe extraction protocols. Physical and electric methods have been proposed as alternative methods. Ultrasonic-assisted extraction is one of the important techniques for extracting valuable compounds from vegetal materials. Resveratrol extracted from grape by ultrasonication was found to increase by 24–30% compared with the conventional solvent extraction [2]. Electrically induced extraction technologies (pulsed electric field (PEF) and high voltage electrical discharge (HVED)) were also tested for extraction of soluble materials from the cellular tissues. Based on electroporation mechanism, PEF causes transformation and rupture of cell membranes, which facilitates the release of intracellular products. PEF has been demonstrated to improve mass transfer in extraction of different compounds such as sugar from apple, or red beetroot pigment [3, 4]. As a more “destructive” treatment, HVED can create electric arc in liquid, pressure shock waves, bubbles cavitations, and other physical phenomena, which provoke mechanical disintegration of cell walls and cell membranes. This technology has been applied to the intracellular extractions from vegetative raw material, linseed [5], and yeast cells [6]. Recently, HVED was proved very effective for extraction of polyphenols from grape pomace [7].

The traditional purification process of the grape extract is performed by adsorption chromatography. However, this process involves the use of large quantity of solvent, which impacts on the process economy and its environmental benignity. Membrane processes offer a very powerful alternative for concentrating and purifying bioactive phenolic compounds from aqueous streams, due to their flexibility and mild operating conditions. Membranes have been used to recover phenolic compounds from extracts of mulberry root cortices, and to concentrate catechins from black tea [8]. Phenolics from grape have been processed by ultra-
and nanofiltration, the phenolic content of retentates were 3 – 6.6 times higher than the ones of the feed after membrane processing [9].

The aim of this paper was to investigate the extraction of polyphenols from grape seeds by three unconventional methods including US, HVED and PEF. The extraction efficiency of different methods was evaluated. The influence of extraction methods on the subsequent ultrafiltration behavior and compositions of permeate and retentate was also studied.

MATERIALS & METHODS

Extraction methods

HVED and PEF treatments are conducted with a high voltage pulse generator provided 40 kV-10 kA discharges (Tomsk, Russia). The electrodes of needle-plate geometry are used for HVED (Fig.1a) and two plate electrodes with distance of 1 cm are used for PEF application (Fig.1b). The ultrasonic extraction is performed using an ultrasonic processor (Hielscher GmbH, Stuttgart, Germany) (Fig.1c). The treatment is conducted with grape seeds (50.0 ± 0.1 g) and distilled water (the liquid-solid ratio, L/S=5, w/w) at T= 50°C.

Membrane process

After the treatment, the sample is centrifuged for 10 min at 4000 g, and supernatant solution is collected for filtration process. The PVDF membrane (50 kDa) is used. Dead-end filtration scheme is presented in Fig.2, aqueous extracts (35 mL) are put in the filtration chamber. Hydraulic pressure is applied to the elastic diaphragm. Under pressure, the diaphragm pushes aqueous extracts through the membrane. Permeate is collected and monitored over time by computer. The filtration is conducted with volume concentration ratio (VCR) equal to 3. VCR is the ratio of the initial feed volume to the volume of retentate at the end of filtration.

Analytical measurements

Extracts are analysed for color, clarity, soluble solids, pH and conductivity. Color (A_420) and clarity (T_625) are measured by absorbance at 420 nm and transmittance at 625 nm, respectively. Soluble solids are measured using a digital refractometer (PR-101, Atago, USA) at room temperature and expressed as °Brix. The pH was measured by instrument InoLab pH/cond Level 1 (WTW, Weilheim, Germany). Conductivity was measured by instrument InoLab pH/cond Level 1 (WTW, Weilheim, Germany) with conductivity probe WTW Tetra Con 325. The polyphenol content of grape extract prior and after UF was determined by Folin–Ciocalteau method [10]. Gallic acid is used as standard for the calibration curve. Results are expressed as gram of gallic acid equivalent (GAE) per 100 g of dry matter (g_d).
RESULTS & DISCUSSION

Comparative evaluation of extraction methods

The major difference between the three extraction methods has been related to the mechanisms by which they disrupt the cell tissue. PEF and US disrupt cells with less overall destruction of the cell envelope than HVED do. The mechanism of ultrasonic disintegration is associated with cavitation phenomenon. PEF is based on the electroporation of plant cell membrane and improving the mass transfer [11]. HVED acts by the electrical breakdown in water and causes particle fragmentation and cell structure damage. A higher degree of disruption (finer cell debris) means that the subsequent separation will be more difficult. The photographs showing the grape seeds after the treatments are presented in figure 3. After PEF and US treatments, the grape seeds basically maintained their integrity. However, after HVED treatment, more important fragmentation of the grape seed is observed. This reflects the combined effect of different discharge phenomena (e.g., pressure shock waves, bubbles cavitation) on mechanical damage of seeds, disintegration of cell walls, and mixture homogenization.

![Figure 3. Photographs of the grape seeds after treatments](image)

The energy needed to release a specific amount of polyphenols depends on the type of the treatment of the equipment used for seed disruption. Thus the extraction efficiency of the treatments has been analyzed by calculating the amount of polyphenols released and the specific energy $E$ (kJ/kg) supplied. The formula for calculating the specific energy is given in Table 1.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Formula</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>$E = \frac{W \times S \times t}{m}$</td>
<td>$E$: the specific energy (J/kg); $W$ (watts/m²): the acoustic density; $S$ (m²): the surface of the probe; $t$ (s): the ultrasonic time; $m$ (kg): the mass of the sample;</td>
</tr>
<tr>
<td>HVED</td>
<td>$E = \frac{N \times W_0}{m}$</td>
<td></td>
</tr>
<tr>
<td>PEF</td>
<td>$E = \frac{N \times W_0}{m}$</td>
<td>$W_0$ (J): the energy of each pulse (J/pulse); $N$: the number of pulses;</td>
</tr>
</tbody>
</table>

The extraction efficiency is considered in terms of the specific energy needed to release the polyphenols from grape seeds. The amount of polyphenols released as a function of the specific energy supplied for the three studied treatments is given in Fig 4. It can be seen from Fig. 4 that HVED permits the highest and less energy consuming polyphenol extraction, followed by US and PEF.

![Figure 4. Extracted polyphenols as a function of total energy supplied](image)
Concentration of aqueous extracts by membrane process

The filtration is conducted with aqueous extracts obtained by US, PEF and HVED treatments under the same energy consumption (160 kJ/kg). Fig.5a shows the increase of permeate volume with time for the extracts obtained by three studied treatments. The results show that the filtration is rapid for the extracts obtained by PEF and US. However, the filtration is slow for the extract obtained by HVED (Fig.5a). In order to examine the underlying fouling mechanism, the data are re-plotted in conventional coordinates \( t/V \) vs. \( V \). The classical cake filtration models predict a linear behavior for curves \( t/V \) vs. \( V \). This is in a good agreement with the experimental data presented in Fig.5b for grape seed extract obtained by PEF (\( R^2 = 0.9975 \)), US (\( R^2 = 0.9946 \)) and HVED (\( R^2 = 0.9977 \)).

![Figure 5. Filtration behavior of grape extract obtained by PEF, US and HVED: a) volume \( V \) versus time \( t \); b) \( t/V \) vs. filtrate volume \( V \)](image)

In cake filtration operation, the specific cake resistance \( \alpha \) is often used to characterize the hydrodynamic resistance, and its value determines, to a large extent, the efficiency of the filtration process. The specific cake resistance can be calculated by using the following equations [12]:

\[
\frac{t}{V} = k_1 V + k_2
\]

\[
k_1 = \frac{\mu \omega}{2 \Delta P A^2}, k_2 = \frac{\mu R_m}{2 \Delta P}
\]

Where \( t \) is the filtration time, \( V \) is the filtrate volume; \( k_1 \) and \( k_2 \) are the slope and intercept of the curve \( t/V \) vs. \( V \), respectively. \( \mu \) is the liquid viscosity, \( \omega \) is the mass of solid particles deposited per unit volume of filtrate, \( P \) is the filtration pressure, \( A \) is the membrane surface, \( R_m \) is the membrane resistance. The values of \( \alpha \) calculated using Eq. (1) are listed in table 2. The results show that the value of \( \alpha \) is biggest for the filter cake formed from the grape seed extract treated by HVED. Remarkable increase of the specific cake resistance \( \alpha \) is associated with more important seed fragmentation during the HVED treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Specific cake resistance ( \alpha ) (m/kg)</th>
</tr>
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<tbody>
<tr>
<td>PEF</td>
<td>( 8.94 \times 10^{14} )</td>
</tr>
<tr>
<td>US</td>
<td>( 1.25 \times 10^{13} )</td>
</tr>
<tr>
<td>HVED</td>
<td>( 3.20 \times 10^{13} )</td>
</tr>
</tbody>
</table>

Extract quality after membrane process

Table 3 shows the physico-chemical properties of samples obtained after membrane separation. Compared to the feed, a remarkable reduction of the polyphenol content is observed in the permeate fractions (57.0% for PEF, 61.5% for US and 78.3% for HVED, respectively), so significant removals are obtained for these compounds. Considering the molecular weight of polyphenols (290-1200 g/mol), this phenomenon can be explained by the screening effect of the membrane. Consequently, polyphenols remain concentrated in the retentate streams. Color and clarity of permeate are improved after the membrane separation because of the removal of suspended colloidal particles and high molecular weight solutes presented in the juice. The conductivity of the permeates shows a little reduction in comparison with the feed. Reduction of conductivity...
is related to the removal of ionic components from the extract. Total soluble solids (°Brix) are higher in the retentates and there is no significant modification in the pH values after membrane separation.

Table 3: Physico-chemical properties of feed, permeate and retentate for three extraction methods

<table>
<thead>
<tr>
<th></th>
<th>Polyphenol g/L</th>
<th>Clarity %T</th>
<th>Color A420</th>
<th>Conductivity μs/cm</th>
<th>Brix</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PEF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>5.46</td>
<td>38.1</td>
<td>1.345</td>
<td>1185</td>
<td>1.9</td>
<td>4.66</td>
</tr>
<tr>
<td>Permeate</td>
<td>2.35</td>
<td>92.6</td>
<td>0.155</td>
<td>961</td>
<td>1.3</td>
<td>5.08</td>
</tr>
<tr>
<td>Retentate</td>
<td>10.31</td>
<td>4.98</td>
<td>4.9</td>
<td>1632</td>
<td>4.2</td>
<td>4.69</td>
</tr>
<tr>
<td><strong>US</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>9.39</td>
<td>24.8</td>
<td>1.736</td>
<td>1252</td>
<td>2.2</td>
<td>4.70</td>
</tr>
<tr>
<td>Permeate</td>
<td>3.62</td>
<td>93.0</td>
<td>0.173</td>
<td>992</td>
<td>1.4</td>
<td>4.94</td>
</tr>
<tr>
<td>Retentate</td>
<td>21.68</td>
<td>2.27</td>
<td>5.6</td>
<td>1757</td>
<td>5.5</td>
<td>4.67</td>
</tr>
<tr>
<td><strong>HVED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>15.35</td>
<td>2.82</td>
<td>4.95</td>
<td>1795</td>
<td>4.9</td>
<td>5.15</td>
</tr>
<tr>
<td>Permeate</td>
<td>3.33</td>
<td>90.2</td>
<td>0.218</td>
<td>1013</td>
<td>2.1</td>
<td>5.24</td>
</tr>
<tr>
<td>Retentate</td>
<td>39.02</td>
<td>0.01</td>
<td>20.30</td>
<td>3090</td>
<td>10.7</td>
<td>5.00</td>
</tr>
</tbody>
</table>

As shown in Fig. 6, the color of the original extracts prior to filtration is largely related to the type of treatment. After the filtration, retentates show a dark orange color and permeates are nearly achromatous, indicating the high retention of polyphenols by the membrane.

**Figure 6.** Photos of permeates and retentates of grape seed extracts obtained by different treatments (F: Feed; P: Permeate; R: Retentate).

**CONCLUSION**

HVED permits higher polyphenol extraction from grape seeds than US and PEF treatments for the same energy consumption. However, HVED also results in the longer membrane filtration and higher specific cake resistance. The dead-end ultrafiltration of all studied extracts was governed by the cake formation mechanism. High retention of polyphenols in the retentates is achieved. Electrically assisted technologies combined with ultrafiltration demonstrated their effectiveness for the extraction and concentration of polyphenols from grape seeds.

**REFERENCES**


