New extraction method for determination of primary aromas in grape

Luna Maslov Bandić1, Marko Viskić1, Petra Štambuk2

1Dept. of Chemistry, Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia
2Dept. of Viticulture and Enology, Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia.
E-mail: mviskic@agr.hr

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Most of the quantitative studies on glycosidic flavour compounds were performed by the analysis of hydrolytically liberated aglycones since the reference glycosides are not commercially available.

Most methods for extraction of aroma compounds in grape include first isolation of glycosides, followed by enzymatic or acid hydrolysis, and determination of aglycones by gas chromatography (GC).

Sample preparation procedure is of crucial importance for the high-quality outcome of the analysis. In extractions, high degree of selectivity and recovery can be achieved if there is compatibility between physicochemical sample matrix itself and the extraction phase.

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A Box-Behnken design (BBD) consisting of 30 experimental runs with three replicates at the center point were applied.

The results showed that the highest extraction efficiency was obtained with 4 g of grape skins, 100 g L⁻¹ of β-glycosidase.

According to the experimental design, better extraction results were obtained with Endozyme preparation.

Grape samples of cv. Gewürztraminer were obtained from Experiment station Jazbina, University of Zagreb. Grapes were harvested in a state of full ripeness and were frozen until analysis.

Grape skin was manually separated and blended to obtain a homogeneous mixture. The required mass of grape skins were mixed with β-glucosidase in citrate buffer at and incubated at 40 °C.

The mixture was centrifuged, hydrolysates were filtered and subjected to solid phase extraction (SPE). The aroma compounds from the solution were extracted using Lichrolut cartridges.

Analysis was carried out by Agilent 6890 coupled to a selective MSD 5973 on a ZB-Wax capillary column (60 m x 0.32 mm, 0.50 μm).

For determination of optimal extraction conditions, different factors were investigated. The conditions investigated were grape mass skin (4-20 g), mass concentration of β-glycosidase (5-100 g L⁻¹) and time of incubation (2-24 h). Also, different preparations of β-glycosidase were investigated.

Table 1. Independent factors and their levels used in the reponse surface design

<table>
<thead>
<tr>
<th>Factors</th>
<th>Factor levels</th>
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<tbody>
<tr>
<td>Coded levels</td>
<td>-1 0 1</td>
</tr>
<tr>
<td>X1: Phase ratio (mL g⁻¹)</td>
<td>5 15 25</td>
</tr>
<tr>
<td>X2: Enzyme dosage (mg h⁻¹)</td>
<td>5 52.5 100</td>
</tr>
<tr>
<td>X3: Extraction time (h)</td>
<td>2 13 24</td>
</tr>
<tr>
<td>X4: Enzyme preparation</td>
<td>Endozyme Lallzyme</td>
</tr>
</tbody>
</table>

Table 2. Comparison of predicted and observed values

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Enzyme dosage (g L⁻¹)</th>
<th>Phase ratio (g L⁻¹)</th>
<th>Time (h)</th>
<th>Predicted values</th>
<th>Experimental values (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endozyme</td>
<td>100</td>
<td>25</td>
<td>24</td>
<td>2245.47</td>
<td>2183.45</td>
</tr>
</tbody>
</table>

5. Conclusion

A simple and fast method without a lot of sample preparation steps was developed. Direct hydrolysis with commercially available β-glycosidases was done. Qualitative and quantitative analysis of aroma compounds was carried out by gas chromatography coupled to mass spectrometer (GC-MS). Optimization of extraction conditions such as grape mass skin, concentration of β-glycosidase and time of incubation were investigated by Box-Behnken design. Developed method can be used as a valuable and effective tool for fast screening of aroma capacity of grapes.

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