Sustainable exploitation of wine lees for winemaking applications: a circular economy approach

E. Kokkinomagoulos¹, A. Stamkopoulos¹, A.M. Michaelidou¹, A.M. Goula¹, P. Kandylis¹²

¹ Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 235, 54124 Thessaloniki, Greece
² Department of Food Science and Technology, Ionian University, Argostoli, 28100, Greece

Presenting author: ekokkinom@gmail.com
INTRODUCTION

Evolution of world production of fresh grapes and wine

2022 worldwide wine production by continent

- 65.89% Europe
- 20.15% America
- 6.40% Oceania
- 4.39% Africa
- 3.17% Asia

The world vitivinicultural year 2022 in a nutshell

<table>
<thead>
<tr>
<th></th>
<th>2022</th>
<th>Compared to 2021</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vineyard surface (mha)</td>
<td>7.3</td>
<td>-0.4%</td>
</tr>
<tr>
<td>Wine production (mhl)</td>
<td>258</td>
<td>-1.0%</td>
</tr>
<tr>
<td>Wine consumption (mhl)</td>
<td>232</td>
<td>-1.0%</td>
</tr>
<tr>
<td>Wine exports (mhl)</td>
<td>107</td>
<td>-5.0%</td>
</tr>
<tr>
<td>Wine exports value (bn €)</td>
<td>37.6</td>
<td>+9.0%</td>
</tr>
</tbody>
</table>

All-time record high!!

Stable since 2017

(OIV, 2023)
INTRODUCTION

Energy crisis
High inflation
Global supply chain disruption

wine prices
consumed wine
INTRODUCTION

≈ 350 tonnes/km²

13–20% of grape
0.17 kg/L of wine

2–6% of wine
Yeast cells, tartrates, inorganic and phenolic compounds

(Mendivil et al., 2013)
**INTRODUCTION**

Publications on vitivinicultural by-products per year

"Waste prevention should be the first priority of waste management and re-use and material recycling should be preferred to energy recovery from waste”

Web of Science Core Collection, https://www.webofscience.com/wos/woscc/basic-search)
**INTRODUCTION**

### Chemical composition of main vitivinicultural by-products

<table>
<thead>
<tr>
<th><strong>Grape pomace</strong></th>
<th><strong>Wine lees</strong></th>
<th><strong>Vine shoots</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong>: 3.4–5.4</td>
<td><strong>pH</strong>: 3.6–7.2</td>
<td>Moisture: 7.9–8.4% (DM)</td>
</tr>
<tr>
<td>Polyphenols: 0.09–1.36% (DM)</td>
<td>Polyphenols: 0.19–1.63% (DM)</td>
<td>Ash: 3.0–3.8% (DM)</td>
</tr>
<tr>
<td><strong>Dietary fiber</strong>: 19–38% (DM)</td>
<td><strong>Protein</strong>: 14.5–15.7% (DM)</td>
<td>Holocellulose: 64.2–69.6% (DM)</td>
</tr>
<tr>
<td>Total nitrogen: 1.0–1.7% (DM)</td>
<td>Lipids: 5.0–5.9% (DM)</td>
<td>Lignin: 19.3–21.8% (DM)</td>
</tr>
<tr>
<td>Sugars: 15–33% (FM)</td>
<td>Sugars: 3.5–4.8% (DM)</td>
<td>Pentosans: 18.4–23.6% (DM)</td>
</tr>
<tr>
<td>Lipids: 0.4–1.0% (FM)</td>
<td>Tartaric acid: 24.5–24.7% (DM)</td>
<td>Lipids: 2.4–6.7% (DM)</td>
</tr>
<tr>
<td>Ash: 1.8–2.4% (FM)</td>
<td>Ash: 10.5–10.6% (DM)</td>
<td>Protein: 4.0–5.3% (DM)</td>
</tr>
<tr>
<td>COD: 610 g O₂ kg⁻¹</td>
<td>COD: 72–323 g kg⁻¹</td>
<td></td>
</tr>
</tbody>
</table>

DM: Dry Matter; FM: Fresh Matter; COD: Chemical Oxygen Demand

(Kokkinomagoulos & Kandylis, 2020)
Wine lees
“The muddy residue accumulating in wine vessels after fermentation or during the storage of wine, dried or not.”
Mainly contains dead yeast cells and other compounds.

INTRODUCTION

Production of a commercial yeast extract substitute
INTRODUCTION

Industrial production of yeast extract

Yeast cells → Fermentation/growth → Cell disruption → Hydrolysis → Mechanical disruption → Yeast extract autolysate → Amino acids, peptides, nucleotides, Flavor enhancer, N source in culture media → Yeast extract powder
METHODOLOGY

- Naturally-occurring process (self-digestion)
- Cell membrane disruption by endogenous enzymes
- Release of intracellular material

**Autolysis**

- Yeast extract/growth media for yeast and LAB
- Crushing
- Grape must
- Fermentation
- Wine
- Racking/bottling
- Wine lees
- Bottle of wine
METHODOLOGY

Ninhydrin spectrophotometric method

Extraction I: sequential maceration with Acetone/H₂O and MeOH/H₂O
Extraction II: acid extraction with HCl

Whole Wine Lees → Solid fraction → Residual solids I

Centrifugation

Residual solids II → Liquid yeast extract

Phenolic compounds

Extraction I

Tartrates

Extraction II

pH: 4.0 - 9.0
T: 40 - 65 °C
Solid concentration: 50 - 400 g/L

Autolysis

Free α-amino nitrogen formation

(Chira et al., 2009; Lie, 1973; Salgado et al., 2010)
## METHODOLOGY

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimum T (°C)</th>
<th>Optimum pH</th>
<th>T (°C)</th>
<th>pH</th>
<th>Solid concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitinase</td>
<td>40–55</td>
<td>5.0–7.5</td>
<td>52.5</td>
<td>6.50</td>
<td>400.00</td>
</tr>
<tr>
<td>β-glucanase</td>
<td>50–65</td>
<td>4.0–5.5</td>
<td>45.1</td>
<td>5.01</td>
<td>120.94</td>
</tr>
<tr>
<td>protease</td>
<td>40–60</td>
<td>5.0–9.0</td>
<td>59.9</td>
<td>5.01</td>
<td>120.94</td>
</tr>
<tr>
<td>cellulase</td>
<td>40–65</td>
<td>4.0–9.0</td>
<td>45.1</td>
<td>7.99</td>
<td>120.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45.1</td>
<td>7.99</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.9</td>
<td>5.01</td>
<td>120.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65.0</td>
<td>6.50</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>9.00</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65.0</td>
<td>6.50</td>
<td>225.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.9</td>
<td>5.01</td>
<td>329.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.9</td>
<td>7.99</td>
<td>329.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>229.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>120.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>229.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>50.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>329.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>329.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.5</td>
<td>6.50</td>
<td>225.00</td>
</tr>
</tbody>
</table>

T: 40 – 65 °C  
pH: 4.0 – 9.0
RESULTS

Optimization of autolysis duration of treated wine lees

![Graph showing the optimization of autolysis duration of treated wine lees. The graph plots FAN (mg α-amino N/L) against Time (h) with a peak at 10 days. Key conditions: 52.5 °C, pH 6.5, 225 g/L.]
RESULTS

- Higher autolysis yields: mid-low and highest temperatures.
- Increase of pH and solid concentration within the examined ranges appears to negatively affect autolysis efficiency.

Figure 1. Main Effects Plot of temperature, pH and solid concentration on autolysis efficiency of treated wine lees (FAN).
• Max. yield: 0.236 mg α-amino N/g (@45.1 °C, pH 5.01, 120.94 g L⁻¹)
• Max. FAN increase: 476%
• T, T*T and SC: significant factors
• R²=79.86%

Figure 2. Effect of temperature, pH and solid concentration on autolysis efficiency of treated wine lees (FAN).
Quantification of free amino acids in treated wine lees autolysates by RP-HPLC-FL

|     | T (°C) | pH  | SC (g/L) | % FAN increase | FAN (mg α-amino N/kg) | mg/kg | asp | glu | ser | arg | gly | ala | pro | val | ile | leu | lys | his | sum |
|-----|--------|-----|----------|----------------|-----------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 45.1| 5.01   | 120.94 | 476.5 | 236.2 | 3.0 | 3.0 | 5.5 | 14.8 | 7.9 | 3.8 | 12.5 | 2.5 | 0.7 | 2.9 | 16.7 | 5.4 | 78.8 |
| 45.1| 5.01   | 329.06 | 389.8 | 169.9 | 2.4 | 2.5 | 3.8 | 13.3 | 5.2 | 2.0 | 12.2 | 2.7 | 0.4 | 1.9 | 15.1 | 3.7 | 65.3 |
| 45.1| 7.99   | 120.94 | 338.0 | 206.4 | 4.5 | 2.6 | 3.8 | 9.9  | 7.1 | 3.8 | 10.7 | 2.5 | 0.8 | 3.0 | 12.4 | 1.3 | 62.4 |
| 45.1| 7.99   | 329.06 | 330.7 | 153.1 | 2.3 | 2.3 | 2.4 | 10.0 | 5.2 | 2.0 | 8.7  | 1.3 | 0.7 | 2.3 | 11.3 | 1.0 | 49.6 |
| 59.9| 5.01   | 120.94 | 334.6 | 165.8 | 117 | 4.3 | 7.2 | 34.9 | 12.0 | 9.5 | 20.9 | 7.4 | 5.2 | 10.6 | 39.5 | 6.1 | 169.2 |
| 59.9| 5.01   | 329.06 | 390.0 | 170.0 | 14.3 | 5.4 | 7.1 | 37.5 | 12.8 | 10.7 | 19.8 | 7.9 | 4.0 | 12.7 | 51.3 | 4.8 | 188.3 |
| 59.9| 7.99   | 120.94 | 259.1 | 158.2 | 11.3 | 16.4 | 9.6 | 24.6 | 12.2 | 12.6 | 21.4 | 8.2 | 5.0 | 12.3 | 29.5 | 1.3 | 164.3 |
| 59.9| 7.99   | 329.06 | 330.4 | 153.0 | 9.6 | 5.8 | 8.8 | 5.3  | 7.3 | 9.9 | 17.6 | 5.3 | 2.0 | 6.6  | 14.8  | -  | 93.0 |

Initial

<table>
<thead>
<tr>
<th></th>
<th>T (°C)</th>
<th>pH</th>
<th>SC (g/L)</th>
<th>% FAN increase</th>
<th>FAN (mg α-amino N/kg)</th>
<th>mg/kg</th>
<th>asp</th>
<th>glu</th>
<th>ser</th>
<th>arg</th>
<th>gly</th>
<th>ala</th>
<th>pro</th>
<th>val</th>
<th>ile</th>
<th>leu</th>
<th>lys</th>
<th>his</th>
<th>sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.01</td>
<td>120.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.01</td>
<td>329.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.99</td>
<td>120.94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.99</td>
<td>329.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Commercial yeast extract (1 g/L)

|     |     |     |          |                |                       |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|----------|----------------|-----------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 10563.9 | 31657.2 | 14689.0 | 18874.2 | 7465.1 | 34531.4 | 9445.8 | 22818.9 | 13982.1 | 35737.6 | 15544.4 | 680.3 | 215990.0 |
METHODOLOGY

Extraction I: sequential maceration with Acetone/H₂O and MeOH/H₂O
Extraction II: acid extraction with HCl

Whole Wine Lees → Centrifugation → Solid fraction

Phenolic compounds

Extraction I → Residual solids I

Extraction II → Residual solids II

Liquid yeast extract → Autolysis

Tartrates

Ninhydrin spectrophotometric method

pH: 4.0 - 9.0
T: 40 - 65 °C
Solid concentration: 50 - 400 g/L

Autolysis yield:
Free α-amino nitrogen formation

(Chira et al., 2009; Lie, 1973; Salgado et al., 2010)
Optimization of autolysis duration of wine lees

**Treated**

- 10 days

**Untreated**

- 1 day

52.5 °C

pH 6.5

225 g/L
RESULTS

• **Similar pattern** with treated wine lees for pH and solid concentration.

• **Increase of temperature** within the examined ranges appears to **negatively affect** autolysis efficiency.

![Figure 3. Main Effects Plot of temperature, pH and solid concentration on autolysis efficiency of untreated wine lees (FAN).](image-url)
RESULTS

- Max. yield: 0.236 \(\rightarrow\) 1.393 mg a-amino N/g
- Max. FAN increase: 476 \(\rightarrow\) 833%
- pH*pH: significant factor
- \(R^2=91.75\%

Figure 4. Effect of temperature, pH and solid concentration on autolysis efficiency of untreated wine lees (FAN).
ONGOING RESEARCH

- Effect of commercial enzyme consortia addition on autolysis yield.
- Production of yeast extract powder through freeze-drying of autolysates.
- Substitution of commercial yeast extract in synthetic media and effect on winemaking yeast and LAB growth.
Autolysis of wine lees increased free α-amino nitrogen by up to 833%.

Results are promising towards the production of a novel, yeast extract substitute.

Further investigation is required in order to produce a stable powder, with possible uses within the food industry and winemaking.
REFERENCES


The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 6158).