



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

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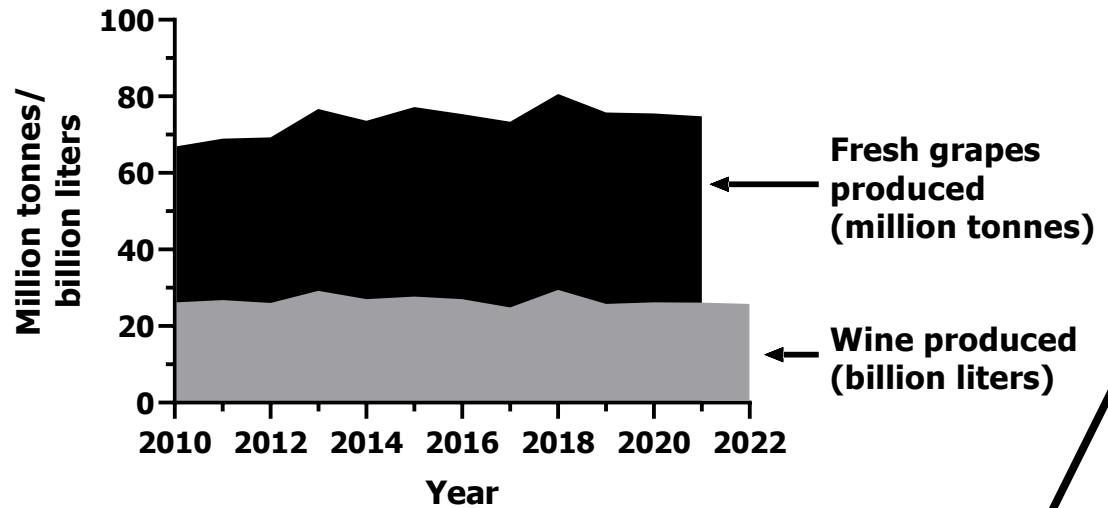
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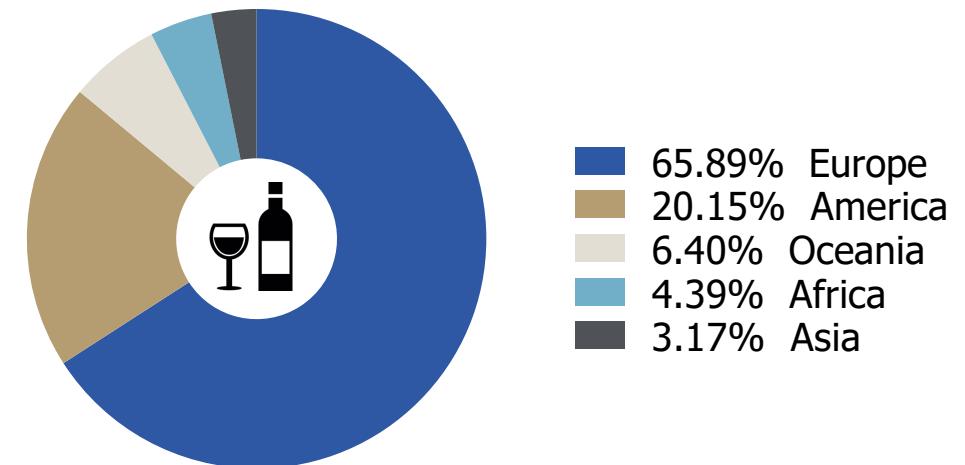
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# INTRODUCTION

Evolution of world production of fresh grapes and wine



2022 worldwide wine production by continent



# INTRODUCTION

## The world vitivinicultural year 2022 in a nutshell



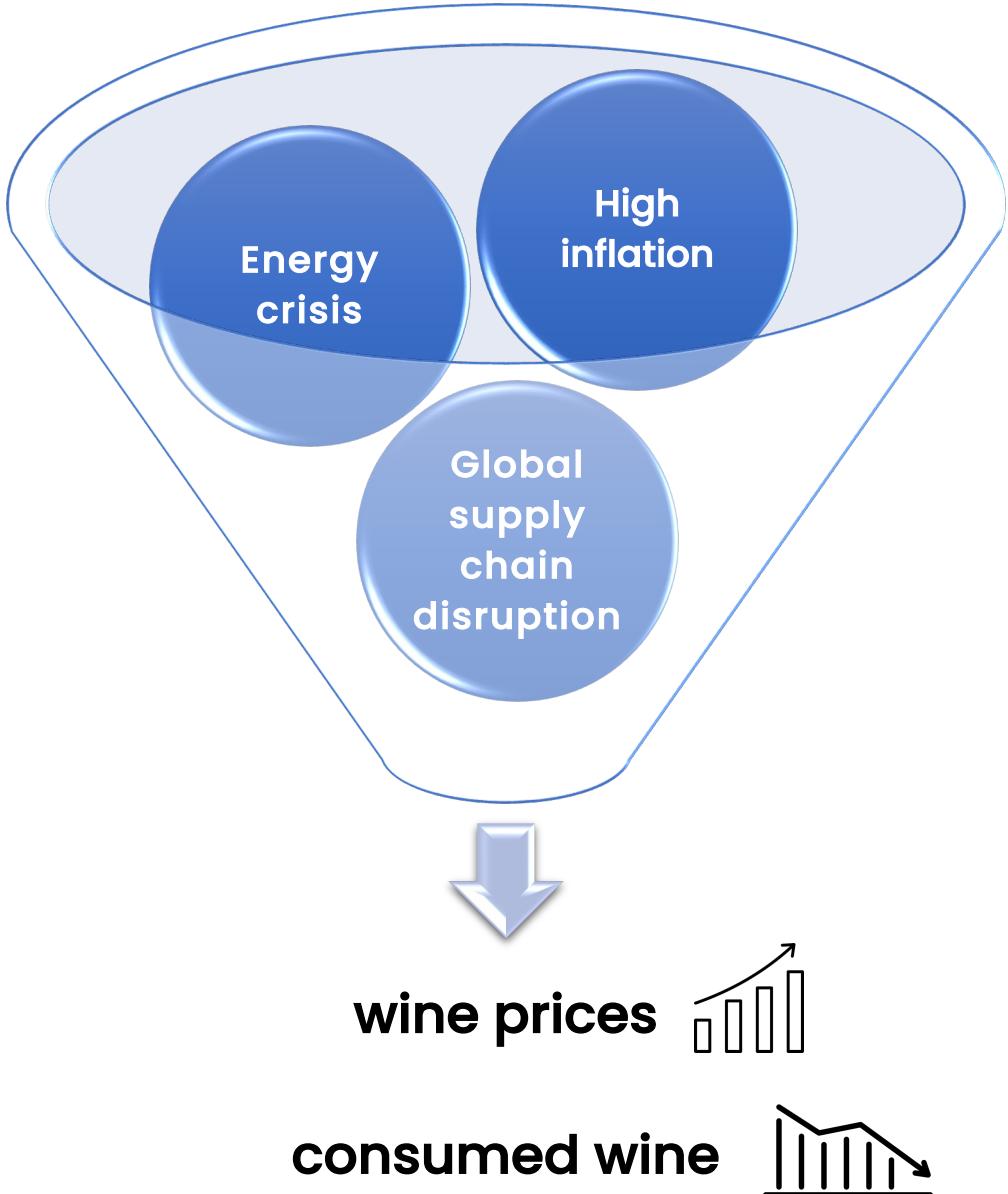
2022      Compared to  
                2021

Vineyard surface (mha)	7.3	-0.4%
Wine production (mhl)	258	-1.0%
Wine consumption (mhl)	232	-1.0%
Wine exports (mhl)	107	-5.0%
Wine exports value (bn €)	37.6	+9.0%

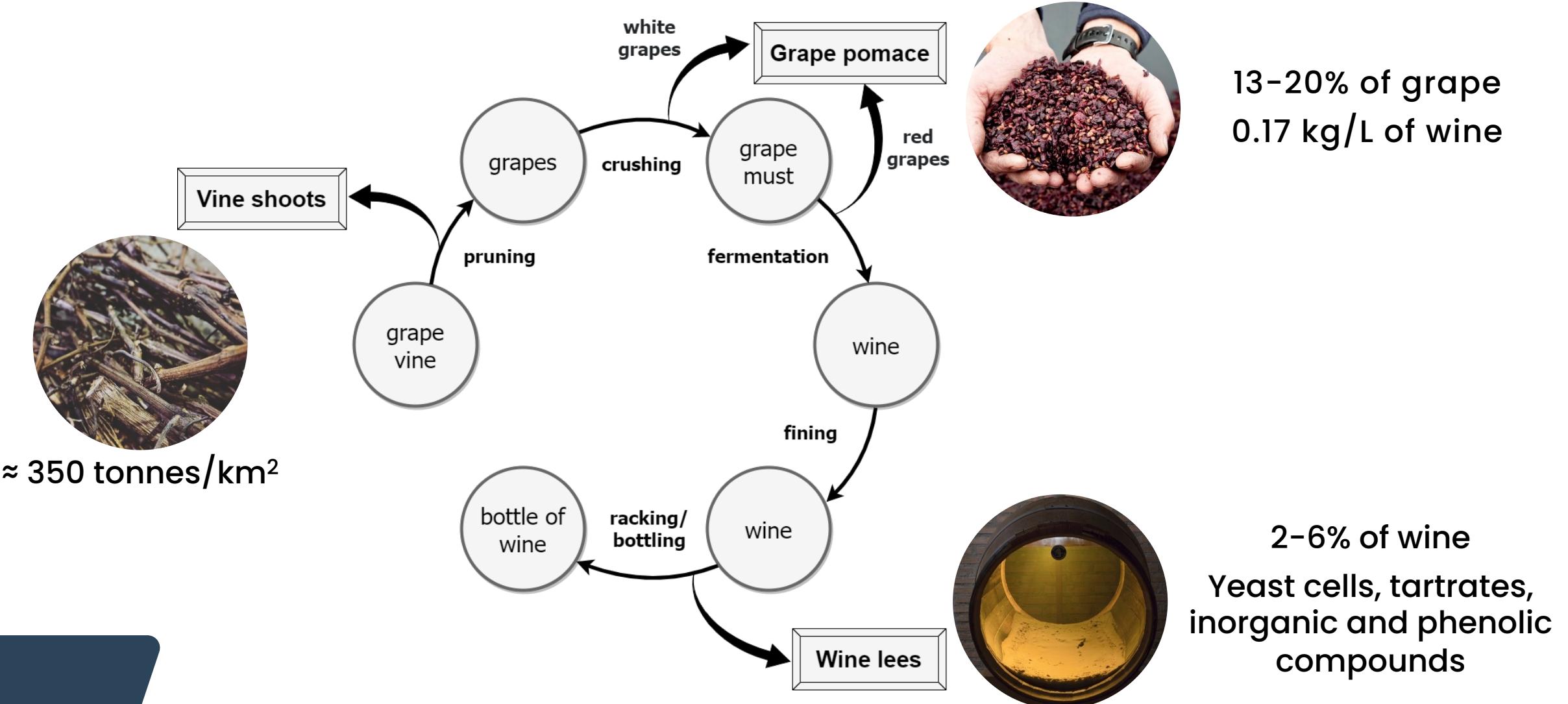
All-time record high!!

Stable since 2017

# INTRODUCTION

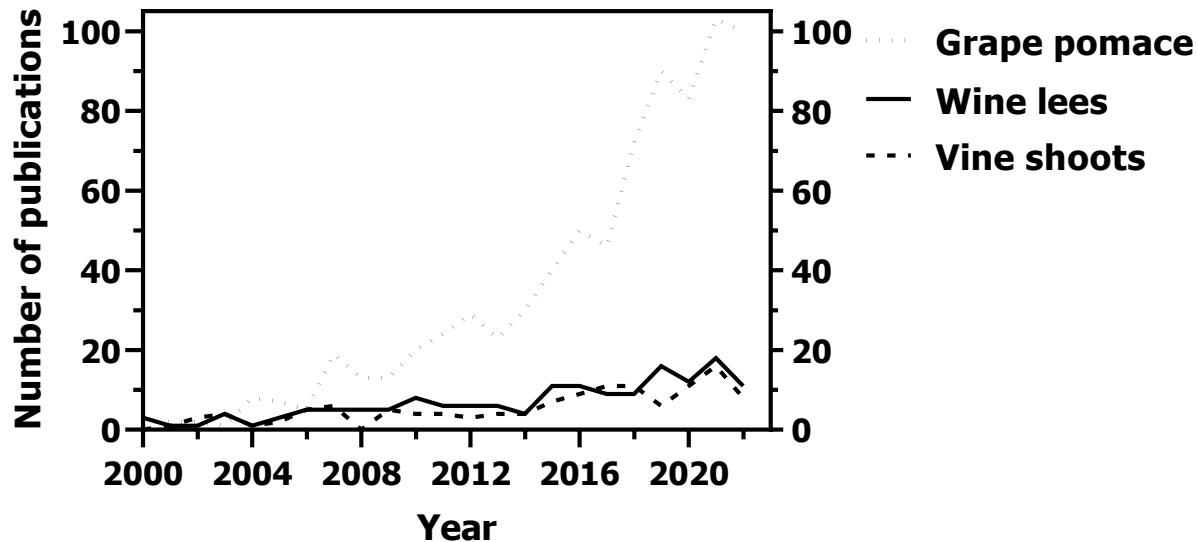


# INTRODUCTION



# INTRODUCTION

## Publications on vitivinicultural by-products per year



## Waste hierarchy



Waste Framework Directive (2008/98/EC):  
"Waste prevention should be the first priority of waste management  
and re-use and material recycling should be preferred to energy  
recovery from waste"

# INTRODUCTION

## Chemical composition of main vitivinicultural by-products

### Grape pomace

pH: 3.4–5.4

Polyphenols: 0.09–1.36% (DM)

Dietary fiber: 19–38% (DM)

Total nitrogen: 1.0–1.7% (DM)

Sugars: 15–33% (FM)

Lipids: 0.4–1.0% (FM)

Ash: 1.8–2.4% (FM)

COD: 610 g O<sub>2</sub> kg<sup>-1</sup>

### Wine lees

pH: 3.6–7.2

Polyphenols: 0.19–1.63% (DM)

Protein: 14.5–15.7% (DM)

Lipids: 5.0–5.9% (DM)

Sugars: 3.5–4.8% (DM)

Tartaric acid: 24.5–24.7% (DM)

Ash: 10.5–10.6% (DM)

COD: 72–323 g kg<sup>-1</sup>

### Vine shoots

Moisture: 7.9–8.4% (DM)

Ash: 3.0–3.8% (DM)

Holocellulose: 64.2–69.6% (DM)

Lignin: 19.3–21.8% (DM)

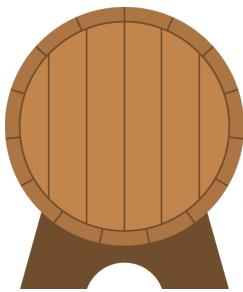
Pentosans: 18.4–23.6% (DM)

Lipids: 2.4–6.7% (DM)

Protein: 4.0–5.3% (DM)

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

# INTRODUCTION



wine  
racking

## Wine lees

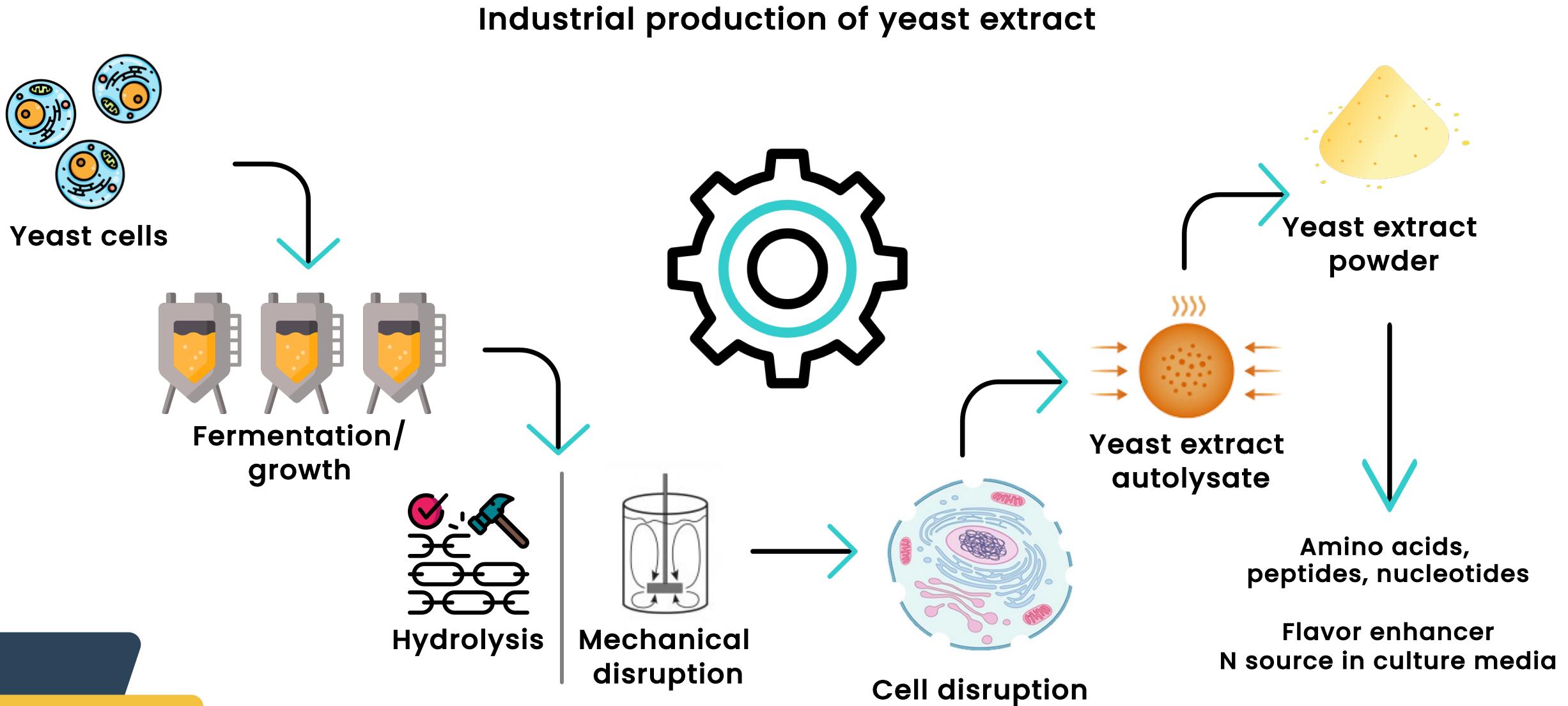
"The muddy residue accumulating in wine vessels after fermentation or during the storage of wine, dried or not"

Mainly contains dead yeast cells and other compounds.

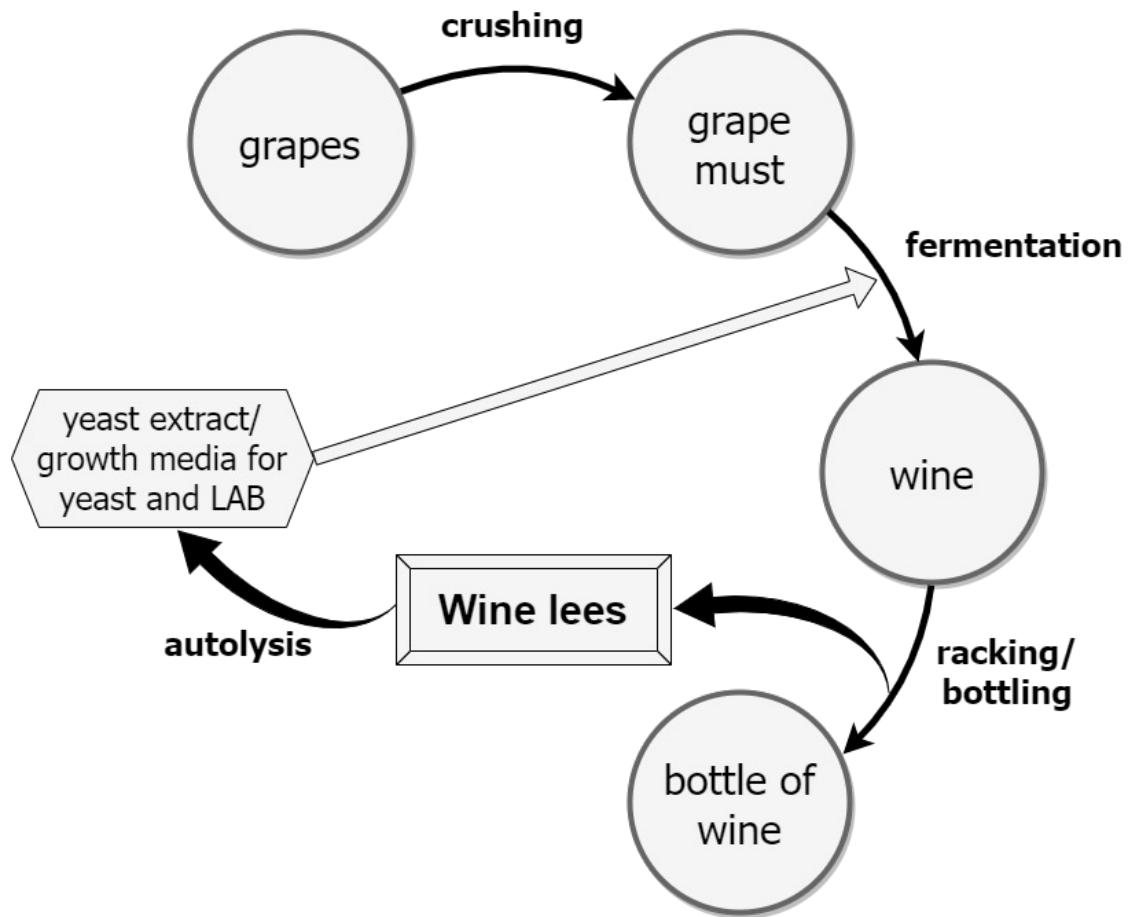
Production of a commercial  
yeast extract substitute



# INTRODUCTION



# METHODOLOGY



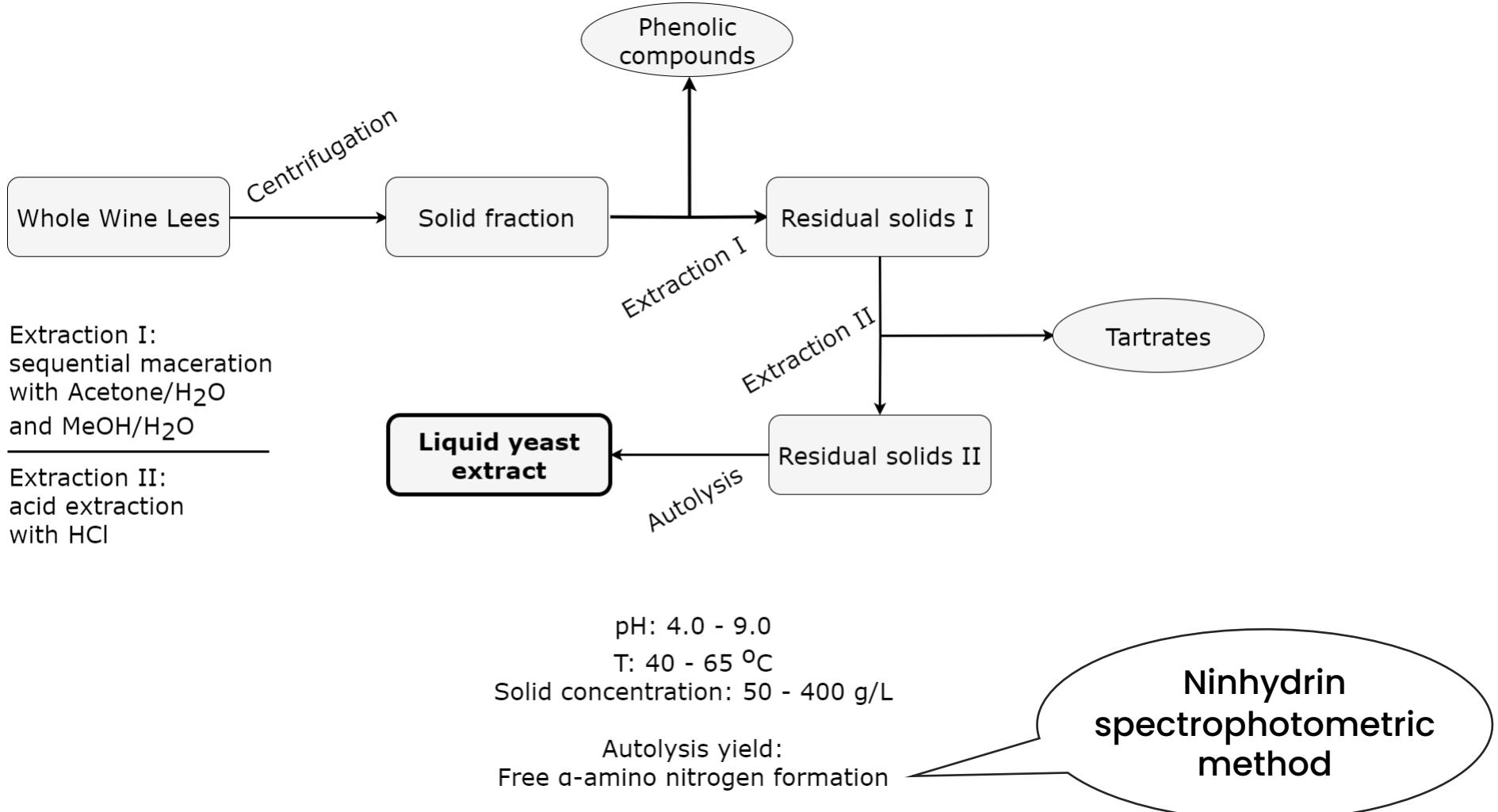
## Autolysis

Naturally-occurring process (self-digestion)

Cell membrane disruption by endogenous enzymes

Release of intracellular material

# METHODOLOGY

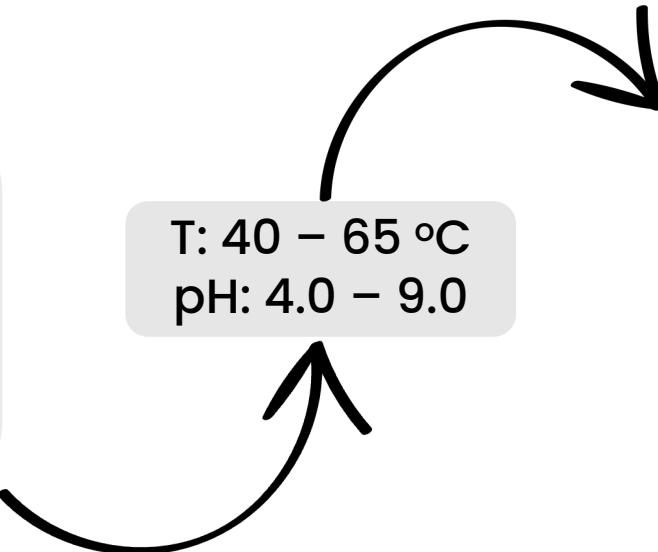


(Chira et al., 2009; Lie, 1973; Salgado et al., 2010)

# METHODOLOGY

Enzyme	Optimum T (°C)	Optimum pH
Chitinase	40-55	5.0-7.5
$\beta$ -glucanase	50-65	4.0-5.5
protease	40-60	5.0-9.0
cellulase	40-65	4.0-9.0

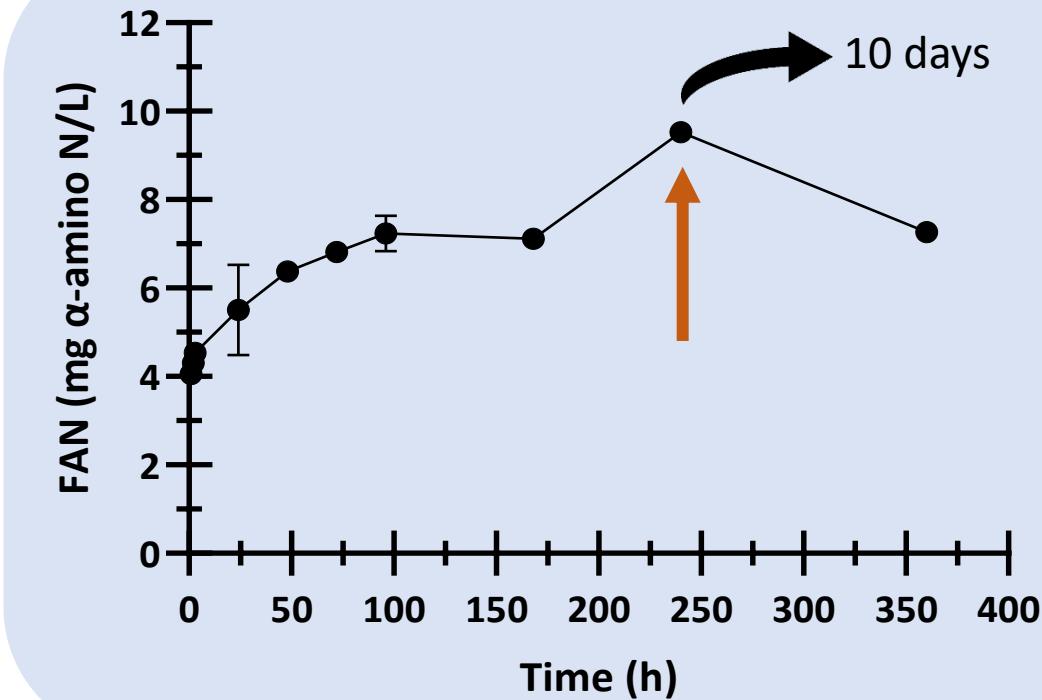
T: 40 – 65 °C  
pH: 4.0 – 9.0



	T (°C)	pH	Solid concentration (g/L)
1	52.5	6.50	400.00
2	45.1	5.01	120.94
3	59.9	5.01	120.94
4	45.1	7.99	120.94
5	52.5	6.50	225.00
6	40.0	6.50	225.00
7	52.5	6.50	225.00
8	52.5	6.50	225.00
9	52.5	4.00	225.00
10	45.1	5.01	329.06
11	59.9	7.99	329.06
12	52.5	6.50	225.00
13	59.9	7.99	120.94
14	52.5	6.50	225.00
15	52.5	6.50	50.00
16	59.9	5.01	329.06
17	45.1	7.99	329.06
18	52.5	6.50	225.00
19	52.5	9.00	225.00
20	65.0	6.50	225.00

# RESULTS

## Optimization of autolysis duration of treated wine lees



# 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.

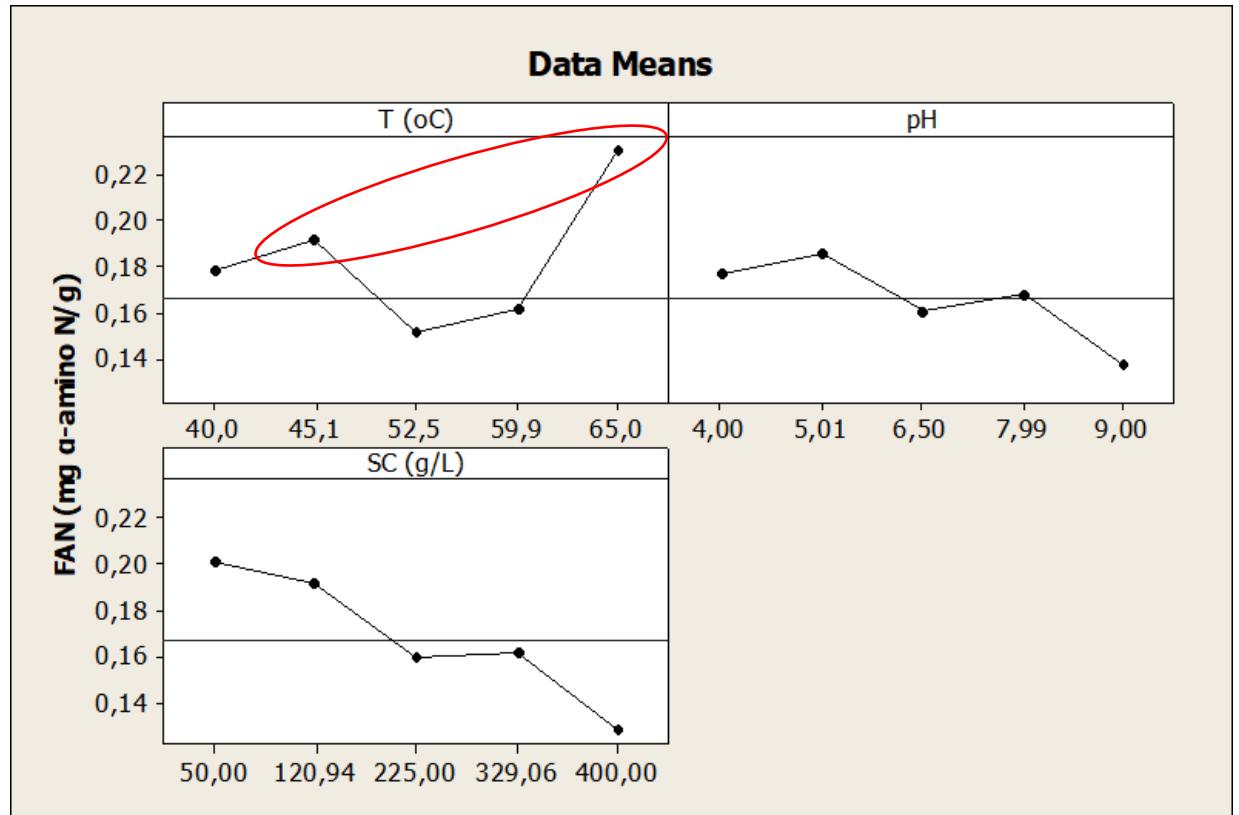


Figure 1. Main Effects Plot of temperature, pH and solid concentration on autolysis efficiency of treated wine lees (FAN).

# RESULTS

- Max. yield: 0.236 mg  $\alpha$ -amino N/g (@45.1 °C, pH 5.01, 120.94 g L<sup>-1</sup>)
- Max. FAN increase: 476%
- T, T\*T and SC: significant factors
- R<sup>2</sup>=79.86%

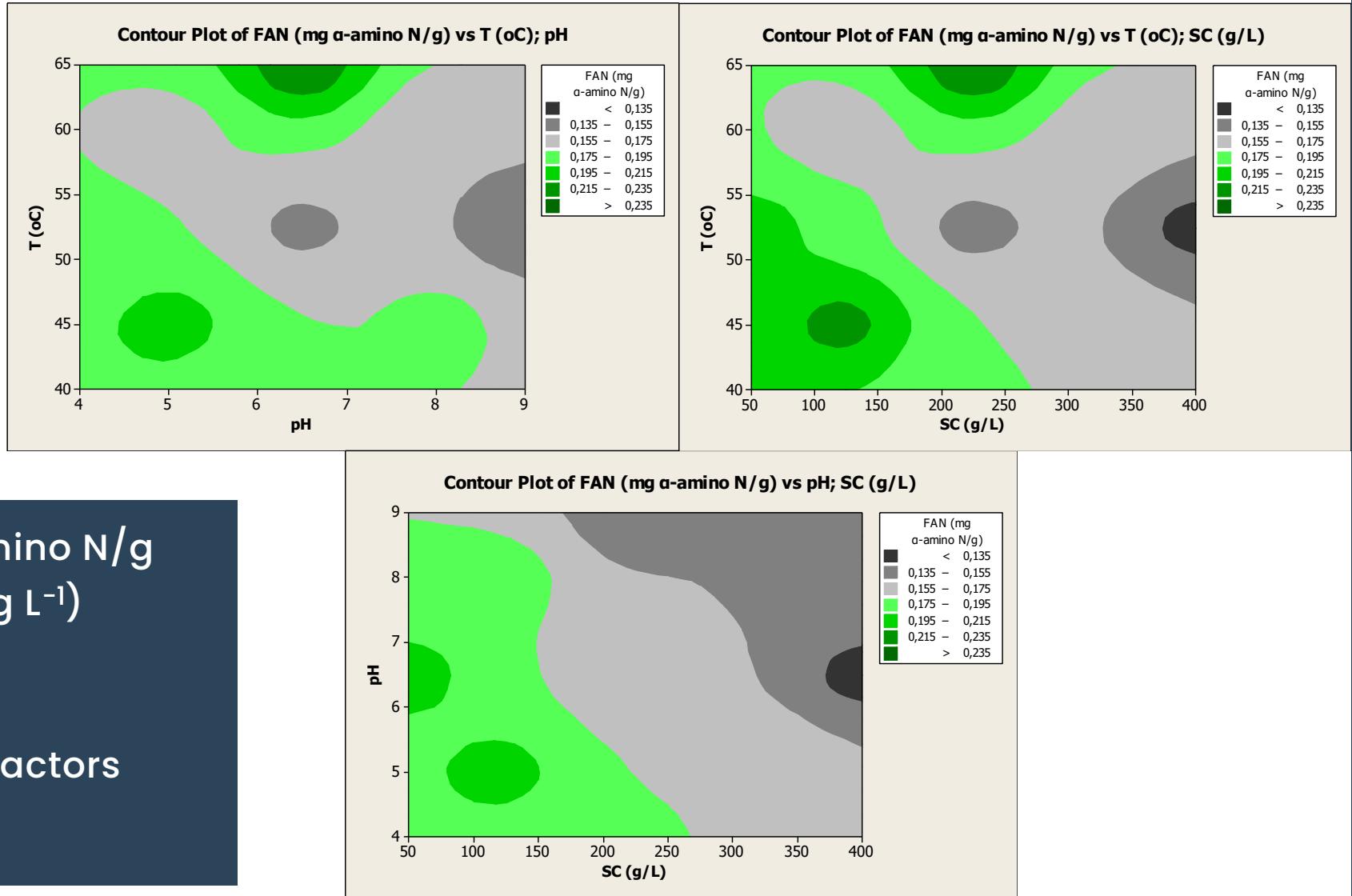


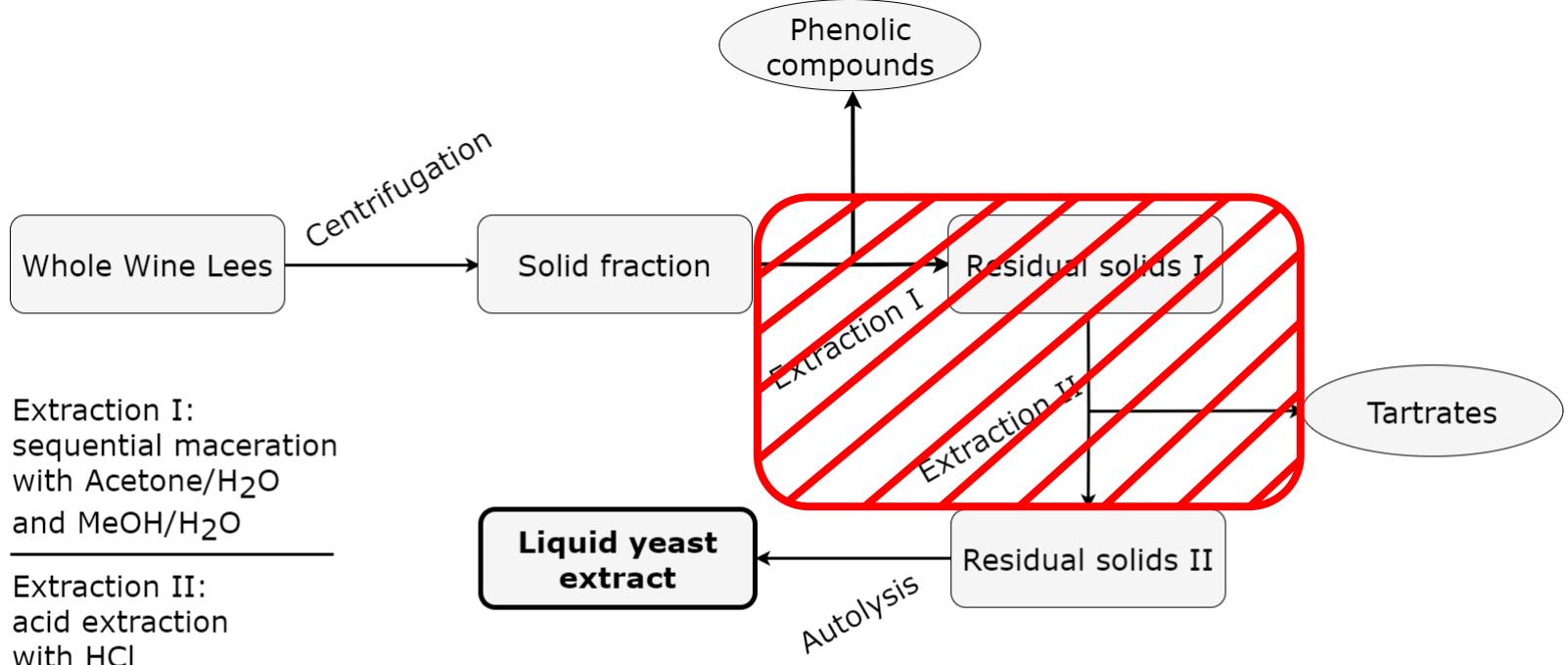
Figure 2. Effect of temperature, pH and solid concentration on autolysis efficiency of treated wine lees (FAN).

# RESULTS

## 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																		
5.01	120.94			49.6	8.2	4.5	5.8	28.8	6.2	11.1	24.3	7.4	3.7	10.4	42.9	7.5	160.9	
5.01	329.06			43.6	7.1	5.1	4.1	29.9	4.8	9.6	21.8	7.9	3.9	9.3	43.9	8.0	155.4	
7.99	120.94			61.1	7.9	5.3	5.7	31.9	6.8	12.0	26.1	8.1	4.2	11.4	43.3	5.3	168.0	
7.99	329.06			46.3	7.1	4.9	4.0	29.4	4.8	9.5	21.6	7.4	3.6	9.5	40.7	6.3	148.8	
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



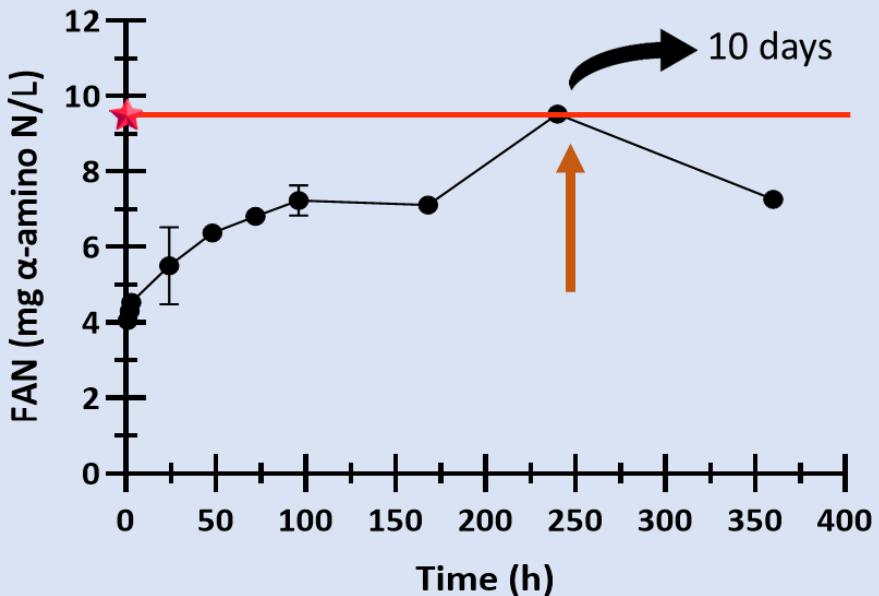
pH: 4.0 - 9.0  
T: 40 - 65 °C  
Solid concentration: 50 - 400 g/L  
Autolysis yield:  
Free α-amino nitrogen formation

Ninhydrin  
spectrophotometric  
method

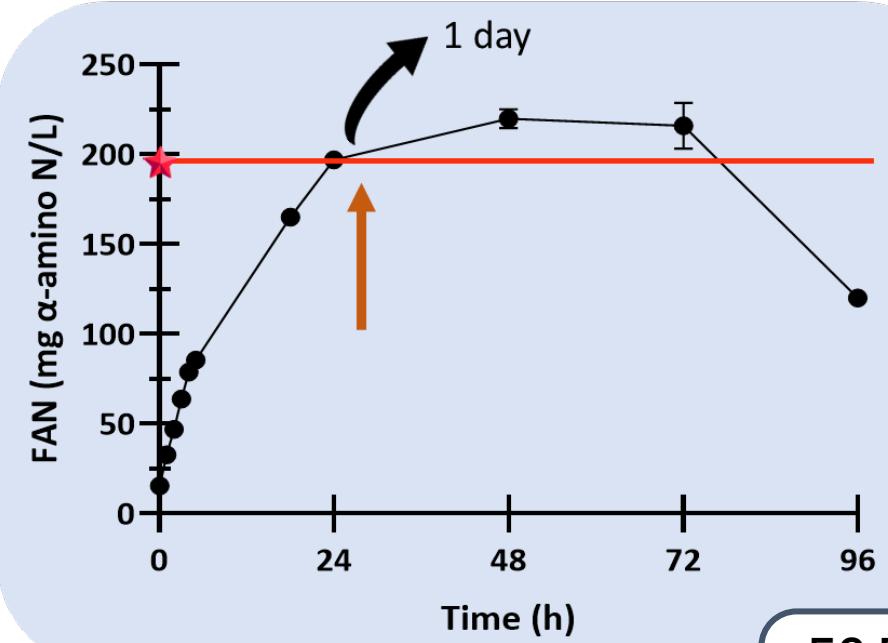
# RESULTS

## Optimization of autolysis duration of wine lees

Treated



Untreated



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.

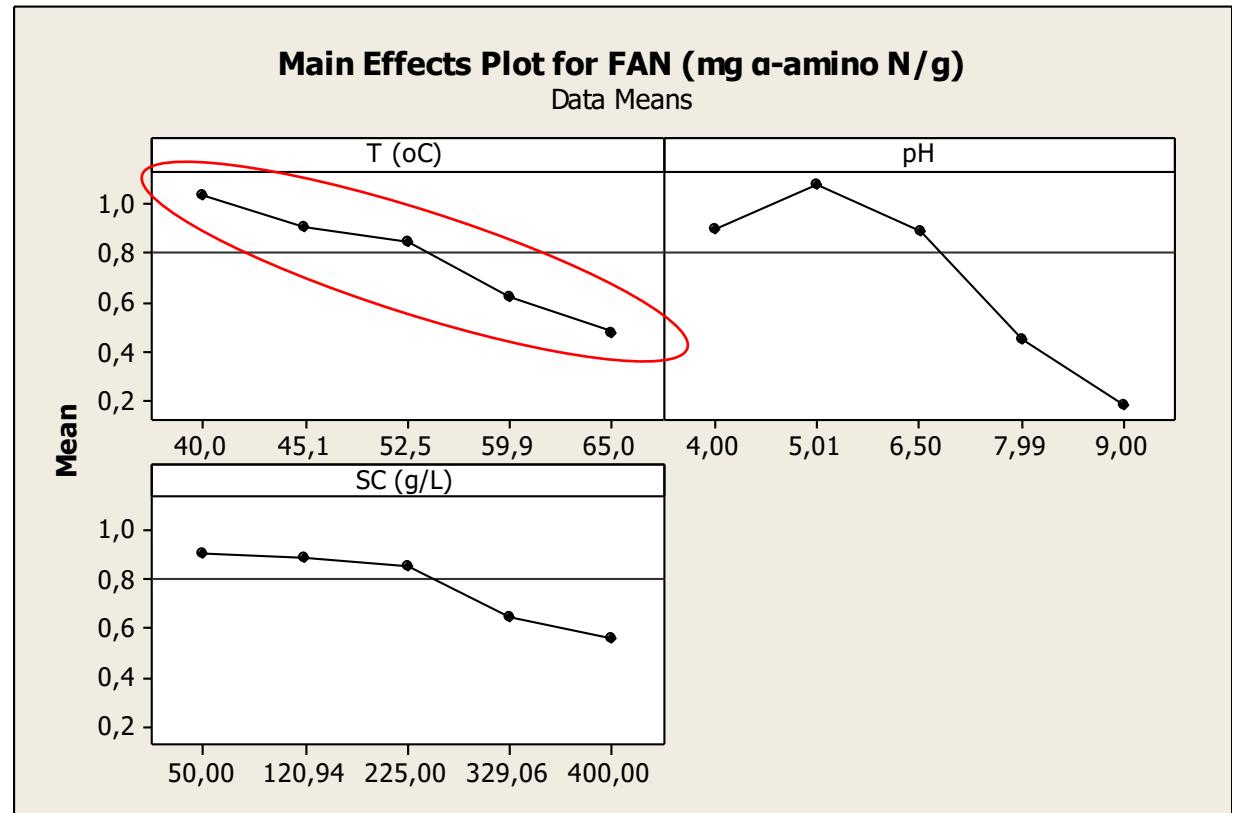


Figure 3. Main Effects Plot of temperature, pH and solid concentration on autolysis efficiency of untreated wine lees (FAN).

# RESULTS

- Max. yield: 0.236 → 1.393 mg α-amino N/g
- Max. FAN increase: 476 → 833%
- pH\*pH: significant factor
- $R^2=91.75\%$

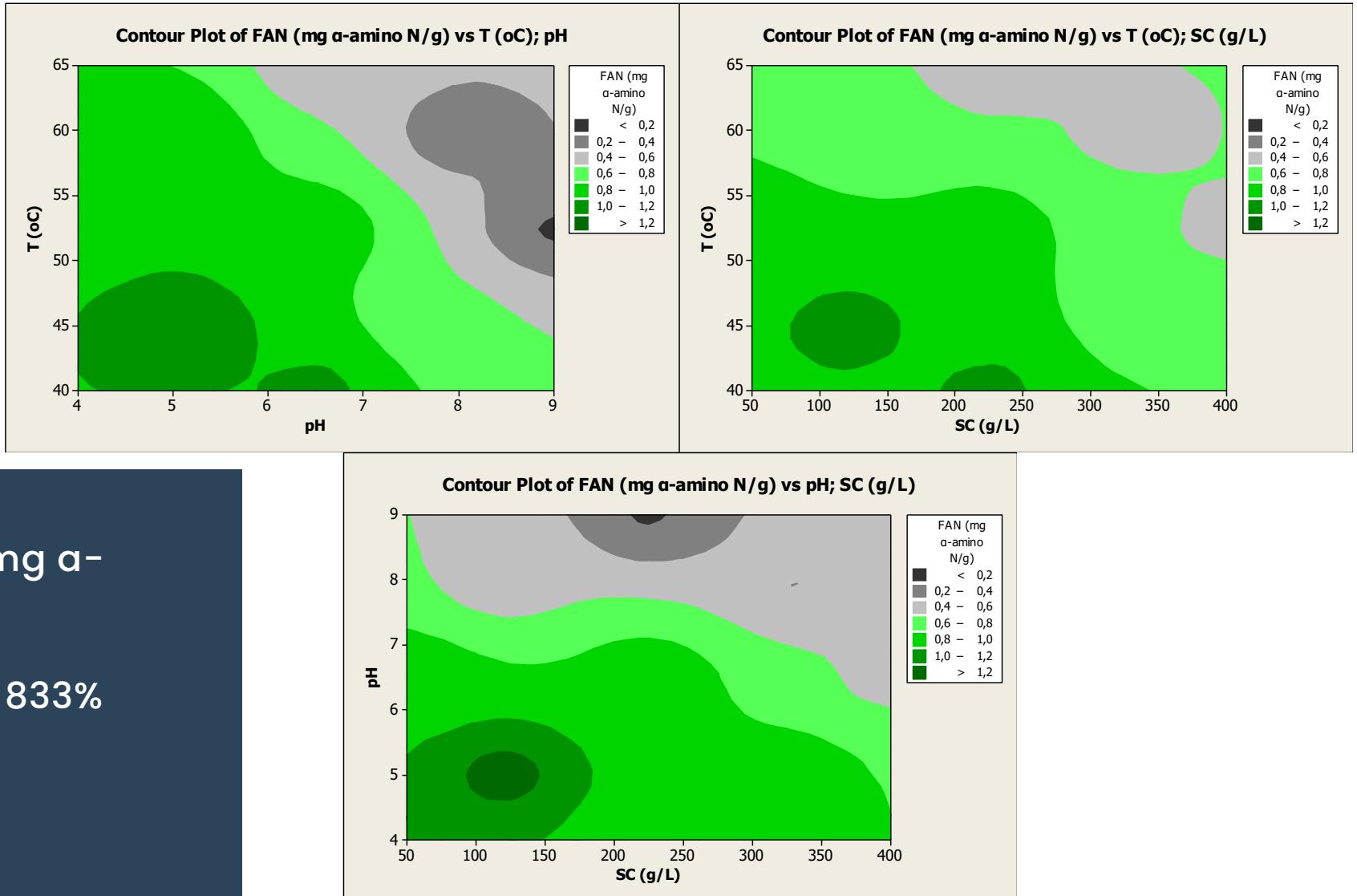


Figure 4. Effect of temperature, pH and solid concentration on autolysis efficiency of untreated wine lees (FAN).

# 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

# CONCLUSIONS

- Autolysis of wine lees increased free  $\alpha$ -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.

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*Thank  
You*



**H.F.R.I.**  
Hellenic Foundation for  
Research & Innovation



ARISTOTLE  
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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).