PRODUCTION OF β-GLUCAN FROM WINERY YEAST WASTE BIOMASS

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Introduction

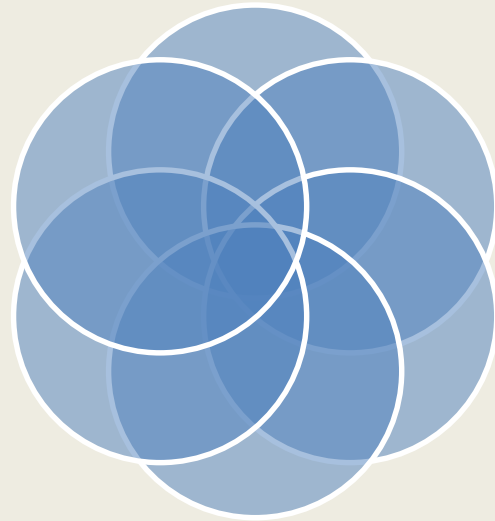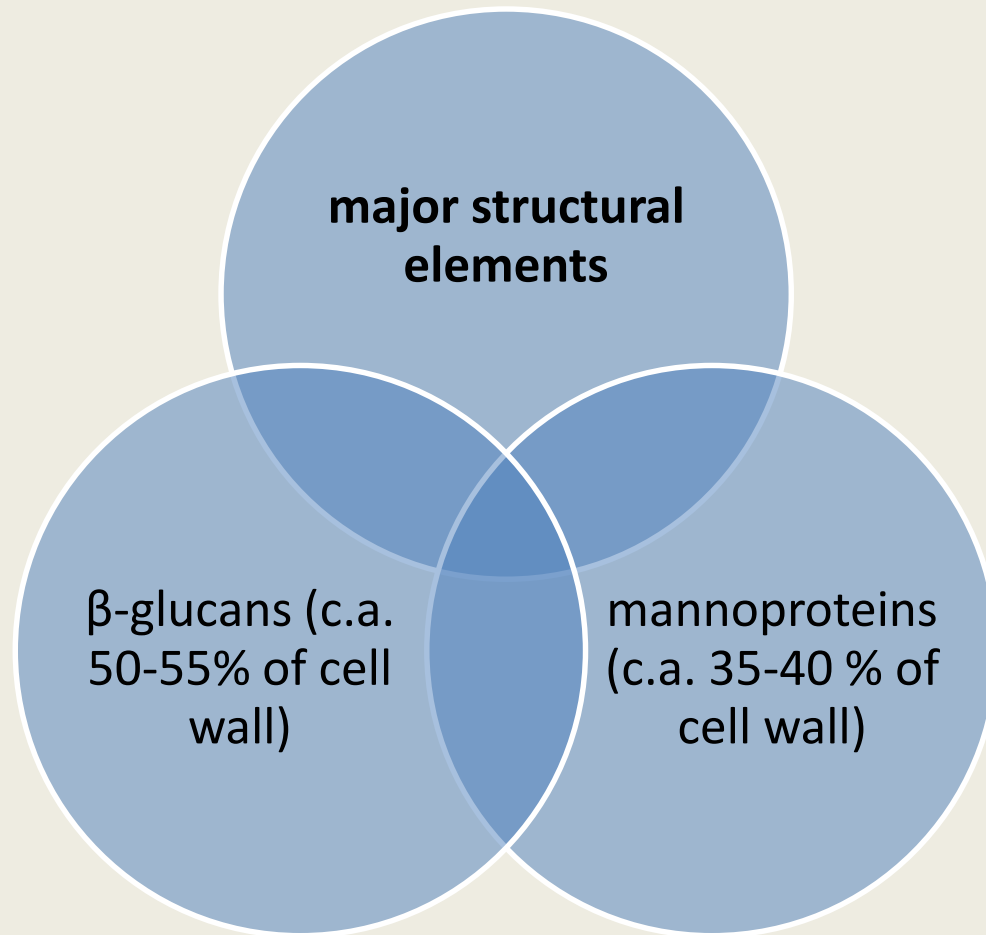**β-Glucan is a widespread molecule in the cell wall of many organisms**

Plants

Yeast

Algae

Bacteria

Fungi

Yeast cell wall

major structural elements

β-glucans (c.a. 50-55% of cell wall)

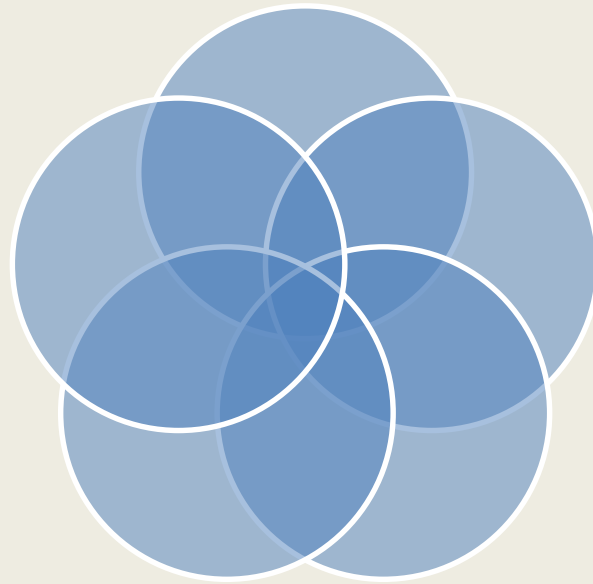mannoproteins (c.a. 35-40 % of cell wall)

minor components

Lipids

α-glucan

Proteins

Chitin

β-Glucan polysaccharide

D-glucose biopolymer

β-1,3-D-glucan, a main component (85%) of about 1500 residues, representing more than 50-55% of cell wall

β-1,6-D-glucan amounts (15%) of about 140 residues, representing 5-10% of cell wall

Last 50 years, more than 2000 studies have been published on the beneficial role of β-glucans in prevention and treatment of a significant number of human and animal diseases and disorders

Antihypertensive

Anticancer-antitumor

Prevention from coronary disease

Strengthen of immune system

Lowering blood cholesterol levels

Lowering of blood sugar index
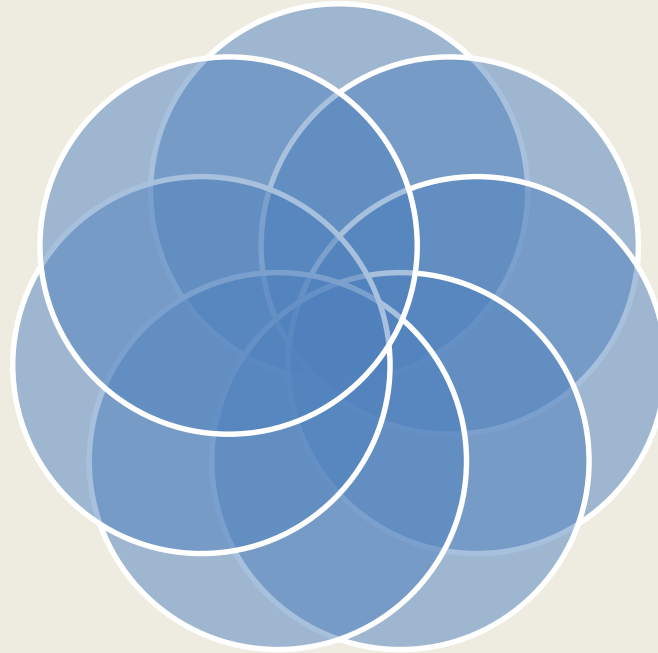
Wine industry

Winemaking process → Formation of a by-product called yeast lees

Wine lees are formed during the

Wine fermentation

Filtration

Centrifugation

Aging steps

Wine lees consist mainly of

spent yeasts

secondary of
bacteria,
phenolics and
pigments (red
wines), tartaric
acid and ethanol

β-glucan production

Yeast cell lysis

physical (sonication, homogenization)

chemical (alkali, acid)

enzymatic (lytic enzymes, glucanases) procedure

Cell disruption

enzymatic
or chlorite
oxidation
procedures
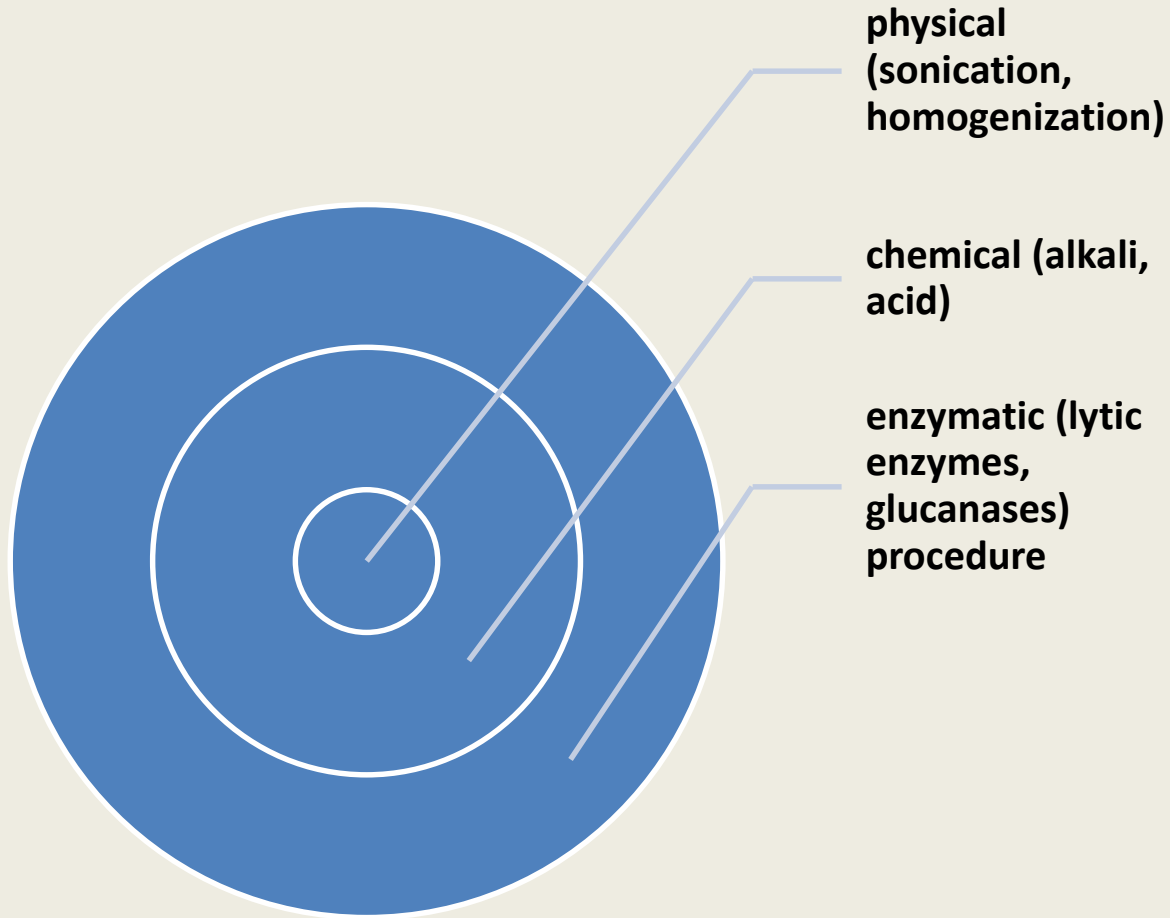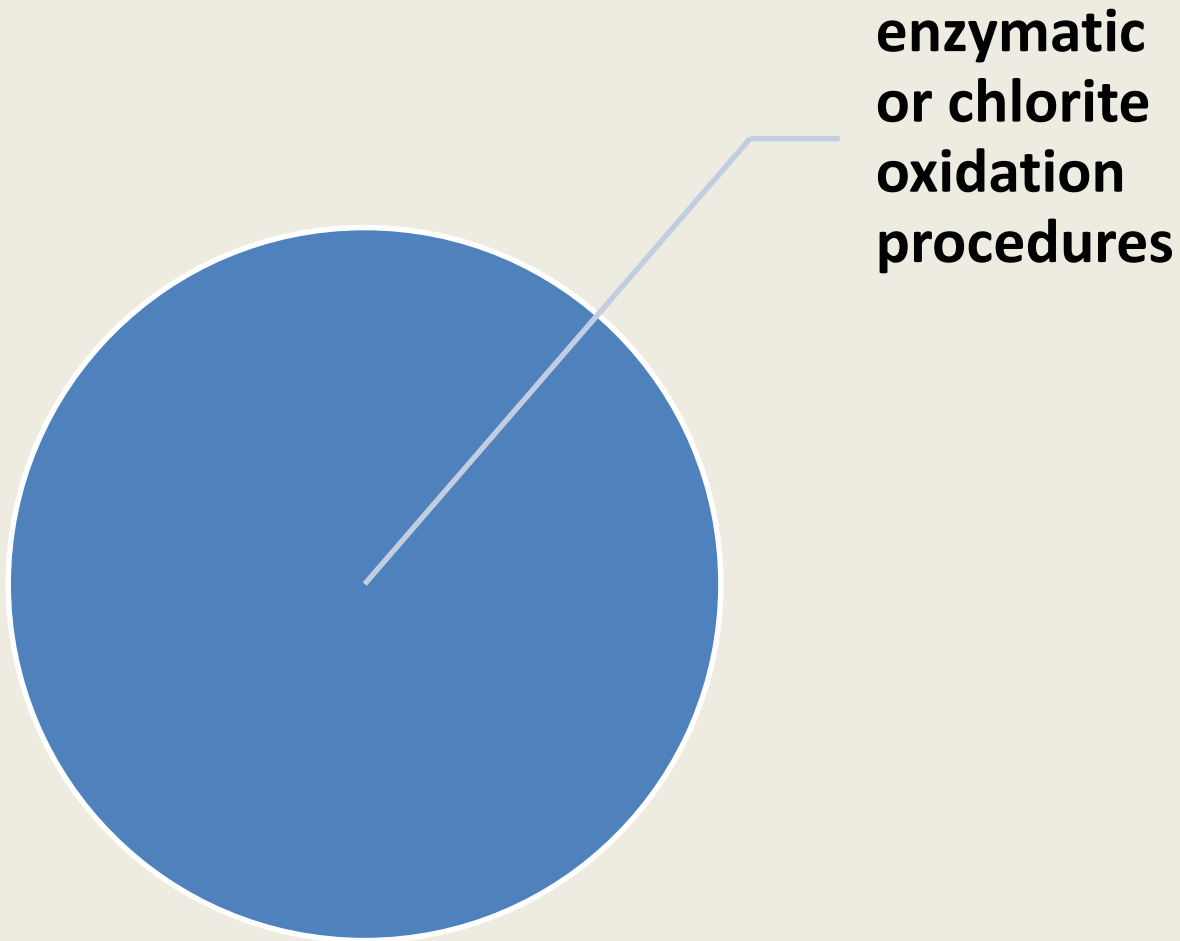
Yeast cell wall purification

Mannoprotein removal → Protein removal → Lipids removal
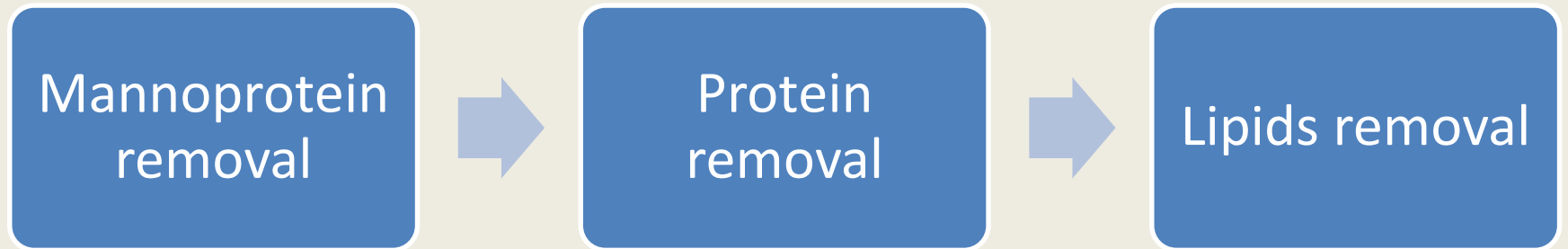
Materials and methods

Yeast cells VIN 13 *S. cerevisiae* strain

Wine yeast lees provided from Nemea and Ritsona region

NaCl, NaOH

Determination of water and total solids of wine lees

Determination of autolysis ratio (R %)

Determination of total phenolic compounds

Determination of β-glucan concentration

Determination of enological parameters of wine
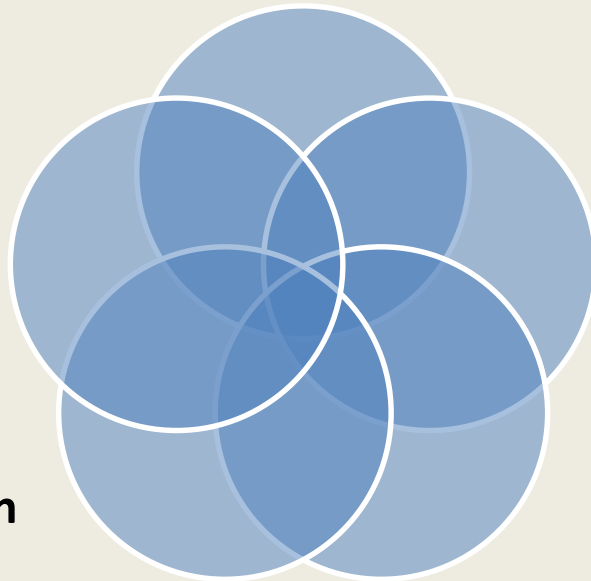
β-Glucan in 2 step extraction process

Yeast cell autolysis with mild conditions (NaCl, Vinegar) → Yeast extract suitable for food applications → Yeast cell walls for β-glucan preparation of food grade → Mannoprotein removal (hot alkali, NaOH)
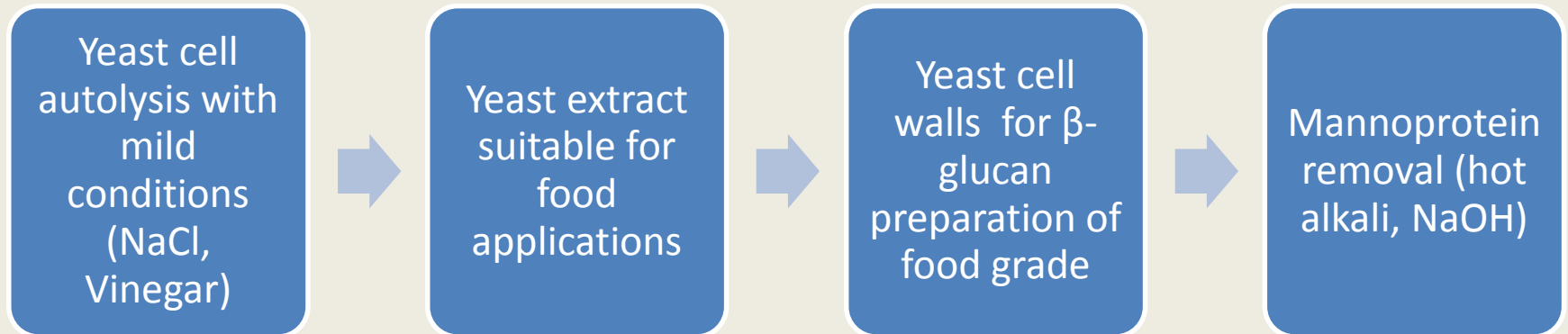
Results and discussion

Yeast autolysis

Optimal autolysis conditions for VIN 13 commercial yeast and winery yeast lees

addition of 3% NaCl as an autolysis promoter

pH 5.0

incubation in water bath at 55°C for 24 h

The pH was set to 5.0 with the use of natural acetic acid and not with HCl as others researchers report

The produced autolysate was a natural product without the use of chemicals or enzymes

The autolysis ratio (R%) was 27,42 % 23.38 % for yeast cell slurry 10% and 20% concentration respectively

These values differ from values reported by other researchers under the same conditions

The differences maybe arise from the different strain that these researchers used and

The conditions under which this stain was cultivated

Also, the differences from other research works can be explained by the use of brewery spent yeast and not winery one

The autolysis ratio of yeast lees was 14.4 %

This can be explained by the fact that at the end of the wine fermentation process, a significant number of yeast cells undergo to enzymatic cell wall degradation autolysis and release of polysaccharides.

Due to that, spent yeast cells after fermentation process are used for the production of wines aged on yeast lees

Yeast β-glucan extraction

VIN 13 *S.cerevisiae* strain
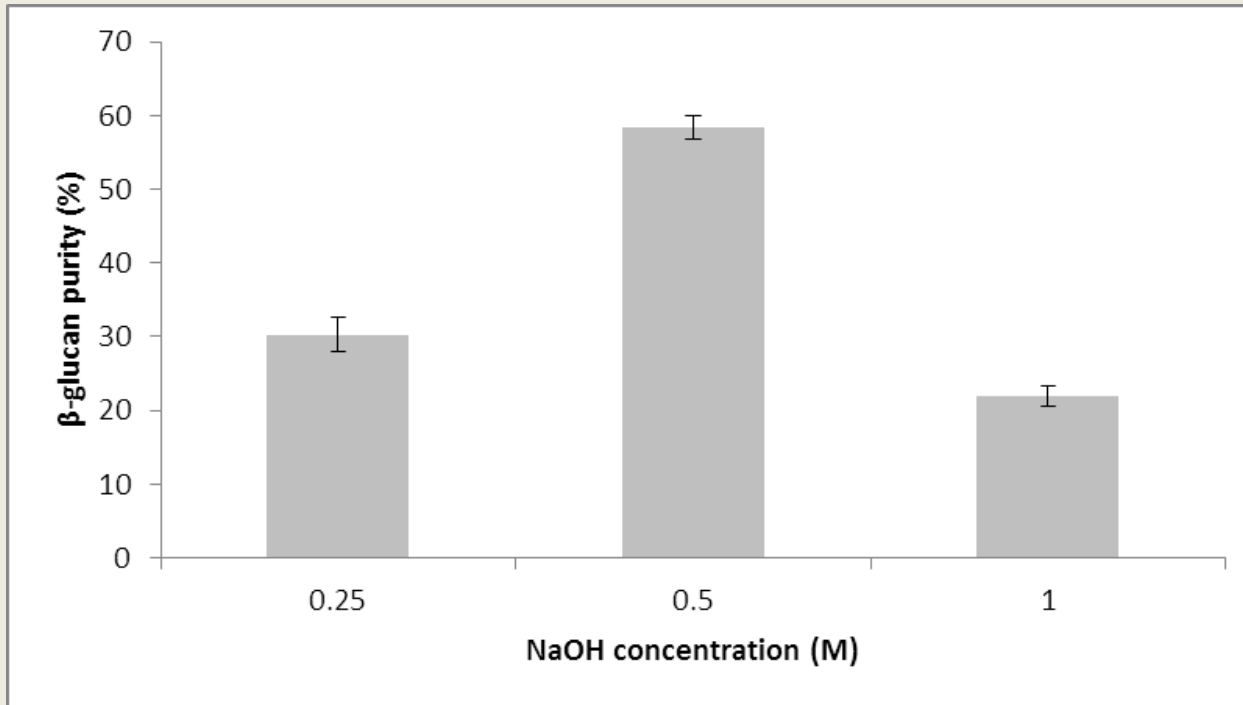

Fig. 2. Preparation of insoluble β-glucan from VIN 13 S. cerevisiae dry yeast with 10% (w/v) initial cell concentration before autolysis, 24 h autolysis, 20% w/v yeast slurry and NaOH 0.25, 0.5, 1 M. Extraction was performed for 1.5 h at 90oC. Error bars represent standard errors of the average value of all replications with each range of β-glucan concentration

- From VIN 13 strain, four different β-glucans were prepared.
- Before alkaline treatment yeast cells slurry in 10% and 20% (w/v) was prepared.
- This was done for the estimation of the appropriate yeast cell concentration which is leading to the most extractable portion of β-glucans.
- For alkaline extraction, two different NaOH concentrations in both prepared yeast slurry were tested for the optimal concentration of NaOH for mannoprotein removal and β-glucan preparation
- This was done for comparative results in NaOH concentration used for β-glucan preparation from pure commercial dry yeast which has not been exposed to the stressed conditions of fermentation process for wine making, as yeast by-product of the fermentation process.

- The extraction time with NaOH for all samples was 1.5 h.

- The 20% (w/v) cell suspension with 0.5 M NaOH gives the highest β-glucan concentration (58.36 ± 1.58 %) in the final yeast powder obtained from the cell wall of VIN 13.

- From the results it is also pointed that the initial cell suspension plays an important role not only during autolysis process for cell wall production but also at the β-glucan preparation with NaOH.

Red wine yeast lees by-product

- The β-glucan extraction from the yeast lees was performed in one step process with the use of hot NaOH solution.

- Alkali extraction solubilizes all yeast cell wall components but β-glucan is received insoluble as sediment

- It can be used for treatment of bulk quantities of yeast waste remaining after fermentation in winery tanks

- The preparation of β-glucan from red yeast lees with NaOH was optimized with four different NaOH concentrations (0.25 M, 0.5 M, 0.75 M, 1 M)

- in four extraction times (0.5 h, 1.0 h, 1.5 h, 2.0 h)

- three different temperatures ($80^{o}C$, $90^{o}C$, $100^{o}C$).

- For the determination of the optimal NaOH extraction conditions, samples of 10 ml from each phase were taken at 0 h, 0.5 h, 1.0 h, 1.5 h and 2.0 h, dried and weight for the determination of yield of β-glucan and the loss of the initial yeast waste biomass during the extraction process

- The main protocol for the optimization of β-glucan extraction was designed for industrial production of β-glucan from winery wastes in such way in order to determine the concentration of NaOH needed for the extraction of β-glucan, the phase at which the recovered β-glucan has obtained the highest purity and the temperature that must be set for the performance of the extraction process.

- Also, in order to determine more precisely the optimal extraction conditions, the β-glucan concentration in initial yeast lees and after autolysis step was measured.

- Induced autolysis had a minor effect on β-glucan extraction.

- This is also in agreement with results with the measurement of total polyphenols (data not shown) in this step and from the determination of autolysis ratio.

- The most purified fraction (19.56 ± 1.28 %) of recovered β-glucan was performed with optimal conditions NaOH 0.25 M, temperature 90°C and extraction time 1.5 h.
- The wine phenolic compounds and the produced alcohol during fermentation inhibit the action of β-glucanase used for clarification of must extracted from grapes affected by Botrytis cinerea
- Due to this fact, it is possible that purity and yield values from red yeast lees treated in the present work for β-glucan determination with enzymatic kit containing 1,3-β-glucanase, 1,6-β-glucanase and chitinase, are higher because of the inhibitive action of absorbed phenols and alcohol from yeast lees on the enzymatic complex
- Commercial products available in the market vary in β-glucan content from 15-90 %
- Generally, β-glucan of about 65% can be achieved but for more purified fractions the procedure is difficult and expensive

- High purified fractions do not mean best results as the immunostimulant activity of β-glucan depends on various parameters (extraction method, degree of brancing, molecular weight, binding with receptors etc.)

- Crude preparations with high β-glucan levels may have adverse effects for humans.

- Different β-glucans appear different effectiveness and we do not precisely know which structural features are the best for inducing its activity

- Differences in β-glucan activity are expected even between various β-glucans differentially isolated from the same source
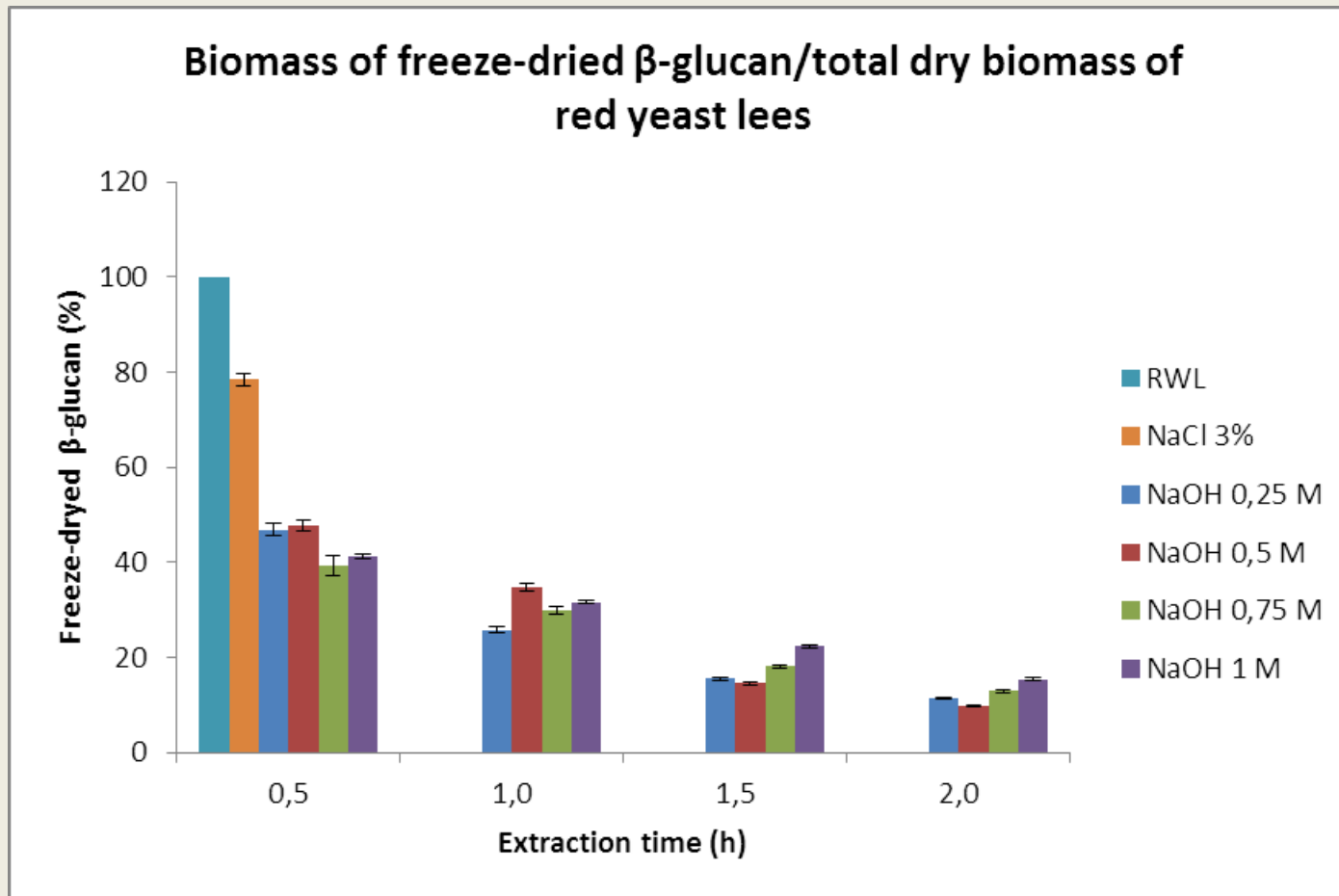
Fig. 4. β-Glucan concentration (%) in dry preparations of insoluble β-glucan from red yeast lees waste biomass with 20% (w/v) cell concentration and NaOH 0.25 M, 0.5 M, 0.75 M and 1 M at extraction time 0.5 h, 1.0 h, 1.5 h and 2 h. RWL is β-glucan content (%) in dry red lees before induced autolysis step. NaCl 3% is β-glucan content (%) in dry yeast lees after induced autolysis. Extraction was performed for 2h at 90°C. Error bars represent standard errors of the average value of all replications with each range of β-glucan concentration.
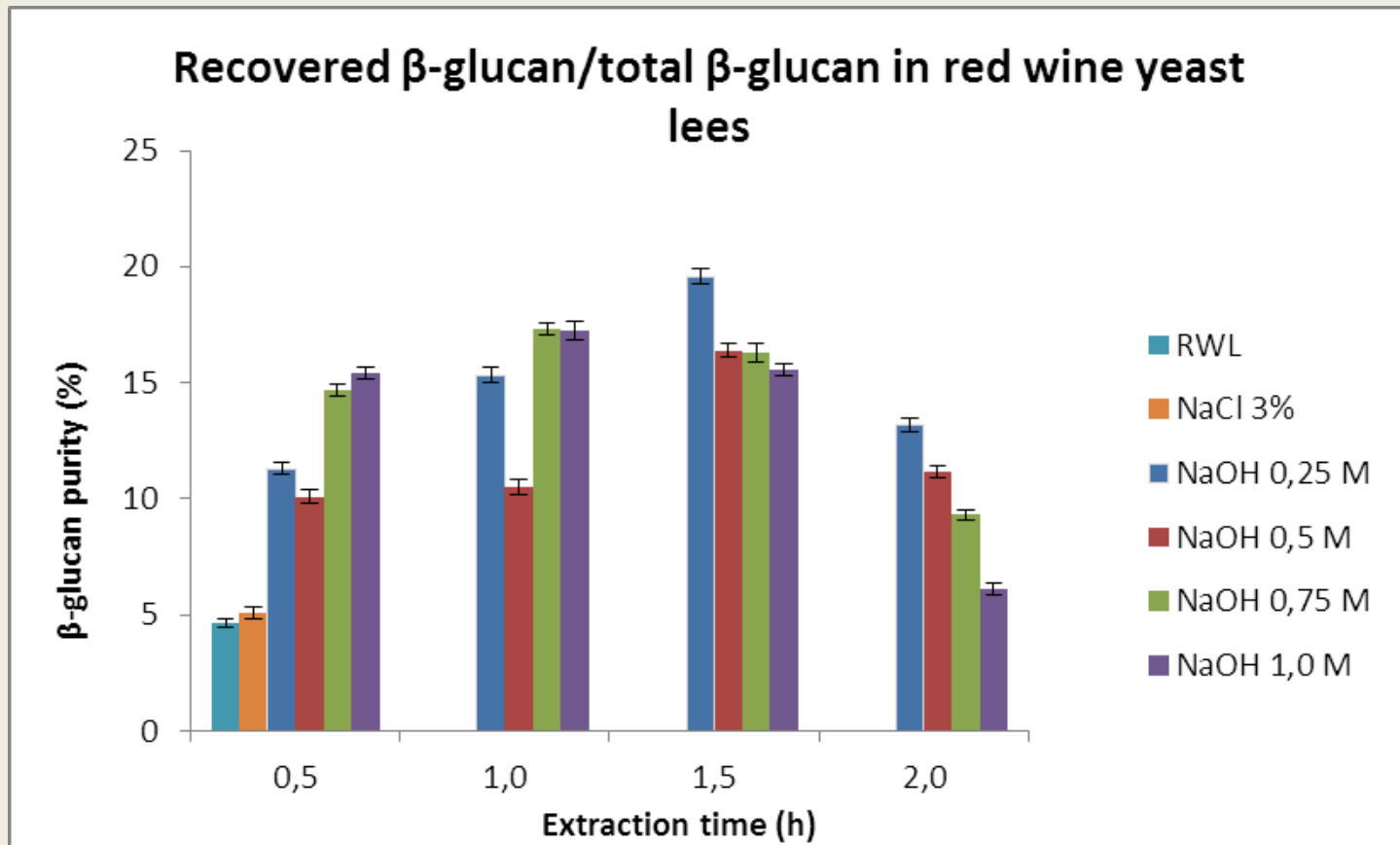
Loss of dry biomass of yeast lees during extraction process

- The freeze-dried β-glucan samples taken every 10 min during the extraction process were weighted and used, apart for β-glucan content determination, also for the loss of initial dry biomass during the extraction process.

- This was done in order to optimize the yield of the extracted β-glucan but also to determine the lost β-glucan content released in the supernatant after NaOH application and the obtained polyphenols (data not shown).

- At the first 0.5 h of the extraction process more than 50 % of the initial biomass was lost as supernatant for all performed NaOH concentrations.

Fig. 5. Dry weight (% of the initial dry biomass) in dry preparations of insoluble β-glucan from red yeast lees waste biomass with 20% (w/v) cell concentration and NaOH 0.25 M, 0.5 M, 0.75 M and 1 M at extraction time 0.5 h, 1.0 h, 1.5 h and 2 h. RWL is dry biomass (control 100%) in dry red lees before induced autolysis step. NaCl 3% is dry weight (%) in dry preparation of yeast lees after induced autolysis. Extraction was performed for 2h at 90°C. Error bars represent standard errors of the average value of all replications with each range of freeze-dried β-glucan concentration.

Yield

- The highest yield 6.34 % ± 1.37 is obtained with NaOH 1 M, extraction time 0.5 h and temperature 90ºC.

- This value is lower than values reported from Zechner-Krpan et al. (13.64 %) and Araújo et al. (10 %) for β-glucan isolated from brewery wastes but wine red lees are structurally more complicated and lower values are expected

Fig. 6. β-Glucan yield (%) in dry preparations of insoluble β-glucan from red yeast lees waste biomass with 20% (w/v) cell concentration and NaOH 0.25 M, 0.5 M, 0.75 M and 1 M at extraction time 0.5 h, 1.0 h, 1.5 h and 2 h. Extraction was performed for 2h at 90°C. Error bars represent standard errors of the average value of all replications with each range of β-glucan yield value.
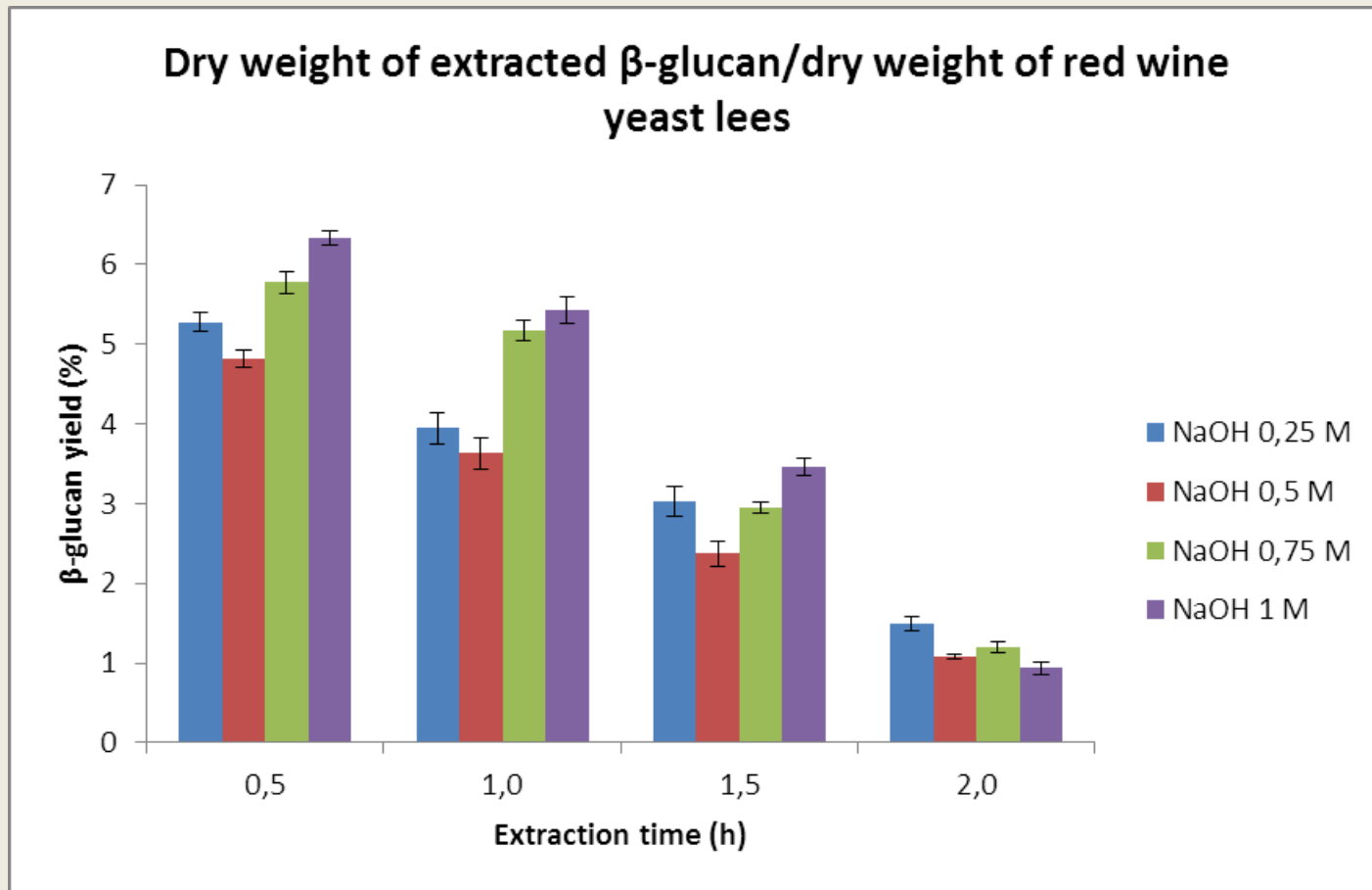
Conclusions

- Yeast waste biomass remaining in the tanks during the wine making process can comprise a new source for β-glucan production.

- Furthermore, the industrial production of added value products, such as β-glucans, from winery yeast wastes justifies with the concept of an intergraded, green and environmentally oriented wine industry.

- Further research in structure, immunotoxicity and immunological activity of recovered β-glucans and applications as additives in functional foods and beverages, is proposed.

- Yeast wine lees, especially red ones, constitute a complex compound which is yet unexplored.

- We suggest that further research in β-glucans with phenolic compounds from red wine lees will offer new perspectives for medical and functional food applications.

Thank you for your attention!