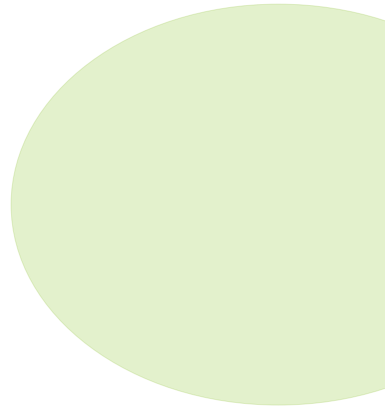
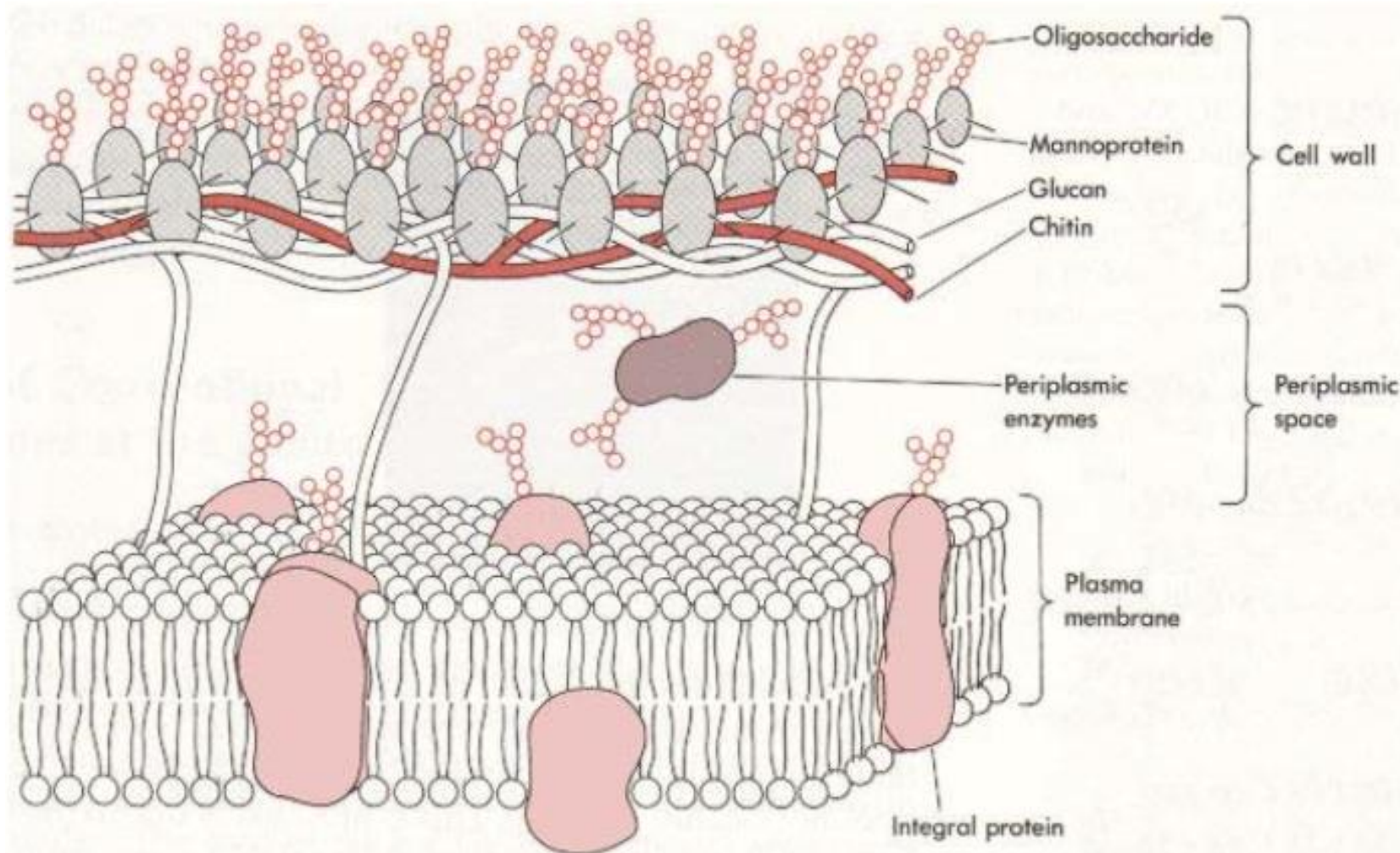


# Analytical methods for mannan and beta-glucan determination in yeast cell wall

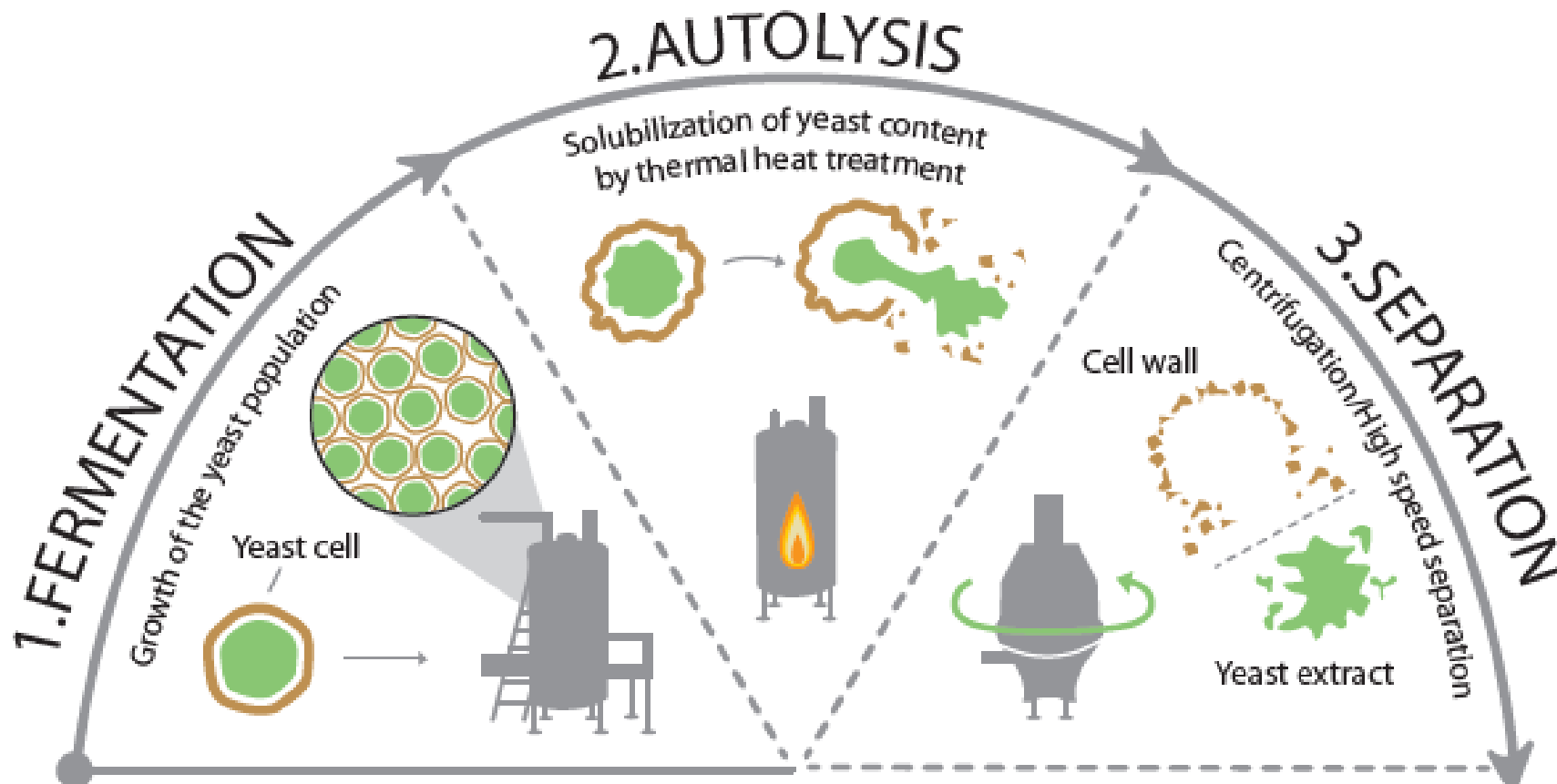


# Yeast Cell Wall Structure



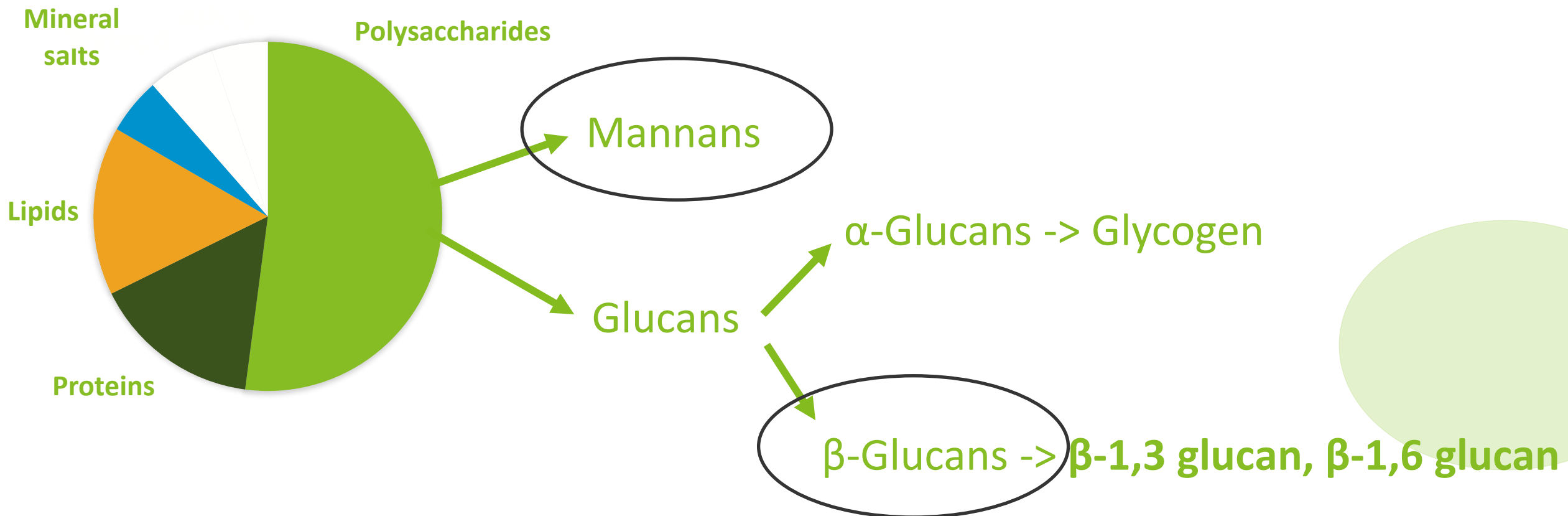


# Manufacturing Process





# Composition of SafMannan – Yeast Cell Wall



**Total glucans = glycogen + beta-glucans**



## Guaranteed parameters

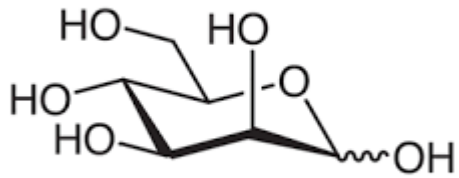
# SafMannan

Mannan	≥ 20%
β-1,3 glucan, β-1,6 glucan	≥ 20%

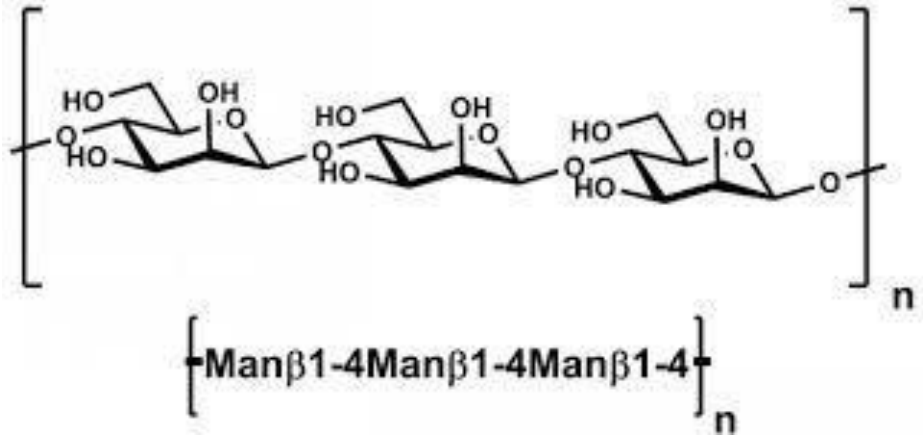


# Composition of Mannan

**Mannose**



**Mannan**

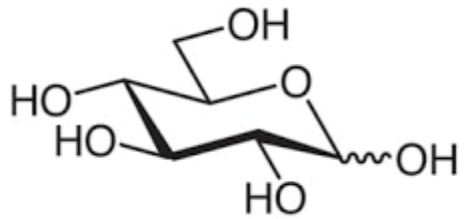


**Mannan is a polymer of mannose units**

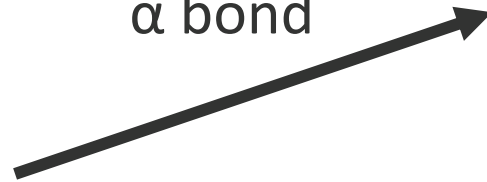


# Composition of Glucan

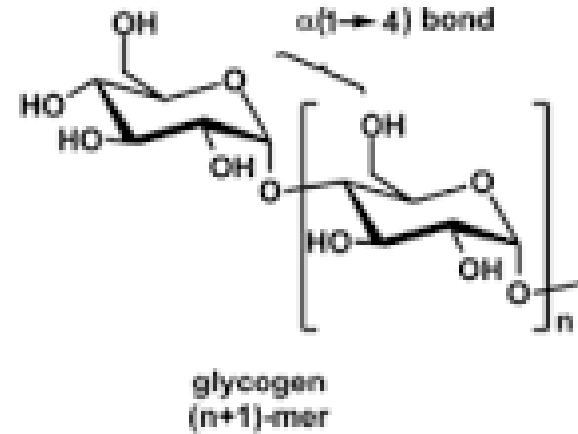
## Glucose



$\alpha$  bond

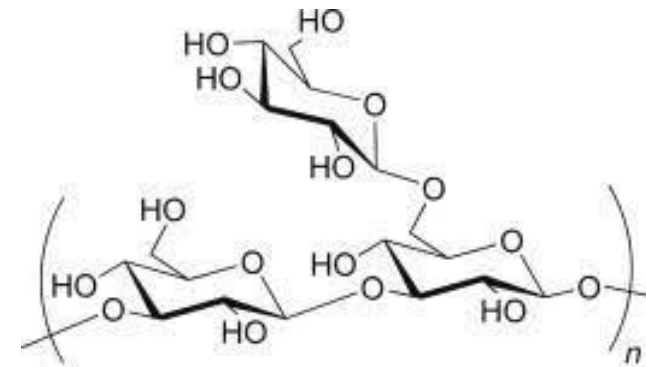


## Glycogen



## $\beta$ -Glucan (1,3) and (1,6)

$\beta$  bond



Glucans are polymer of glucose units



# Mannan

**Objective:**

**Determination of the Mannan content**

**Principle of the method: hydrolysis and dosage of mannose by HPLC**

**Principle of the method:**

- Complete hydrolysis of mannan in mannose
- Dosage of mannose units thanks to calibration curve by HPLC





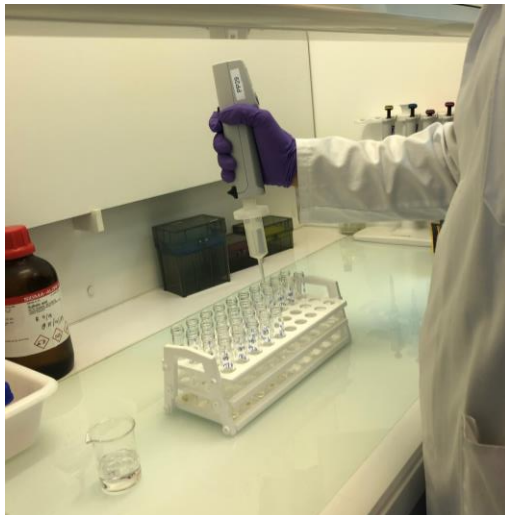
# Mannan

## Objective:

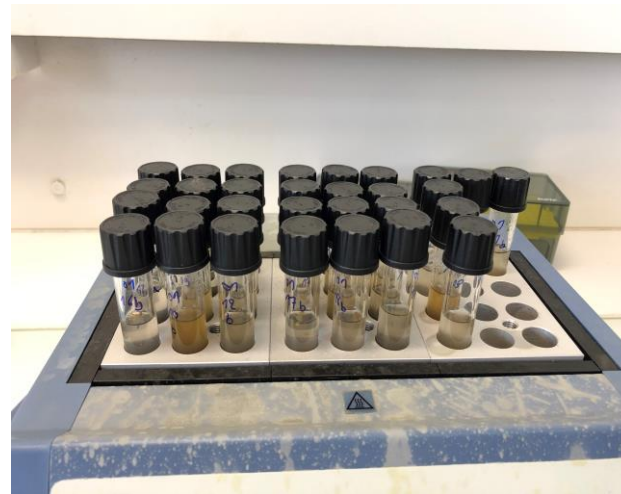
Determination of the Mannan content

Principle of the method: hydrolysis and dosage of mannose by HPLC

## Key steps of the method:



Pre-solubilisation:  
0,5 ml of  $\text{H}_2\text{SO}_4$  72%  
Mix 1h at room temperature



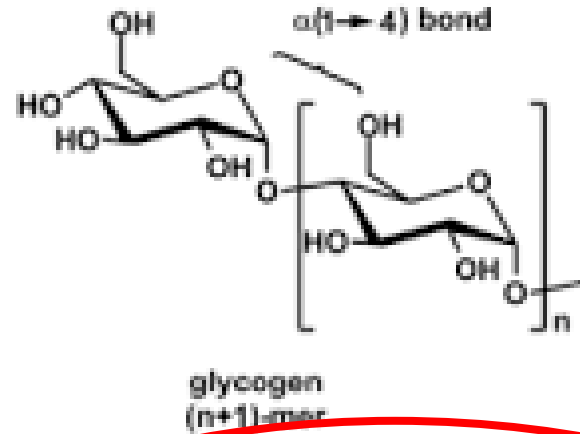
Hydrolysis:  
Addition of water  
**heating at 128°C 1h**  
cooling 10 min in an ice bath



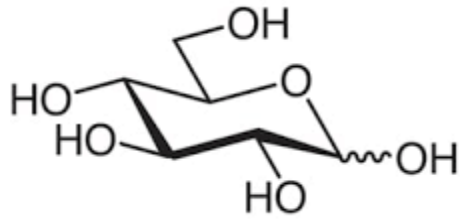
Neutralization with NaOH 32%  
Filtration  
HPLC

# Composition of Glucan

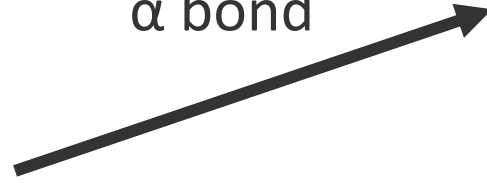
## Glycogen



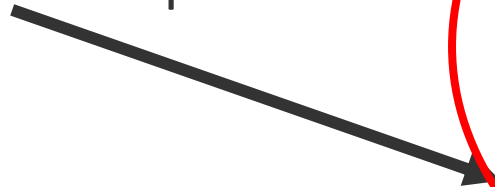
## Glucose



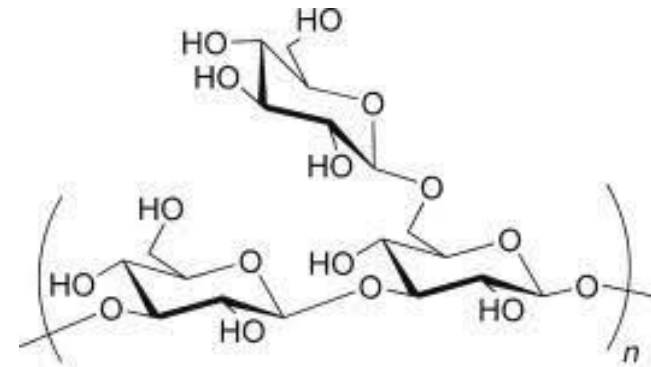
$\alpha$  bond



$\beta$  bond



## $\beta$ -Glucan (1,3) and (1,6)





# Beta-Glucan

**Objective:**

Determination of the beta-glucan content

Principle of the method: enzymatic hydrolysis of beta-glucan and dosage of glucose by HPLC

## Principle of the method:

- **Selective enzymatic** hydrolysis to cleave only the  $\beta$ -(1,3) and  $\beta$ -(1,6) glucan bonds
- Dosage of glucose thanks to calibration curve by HPLC



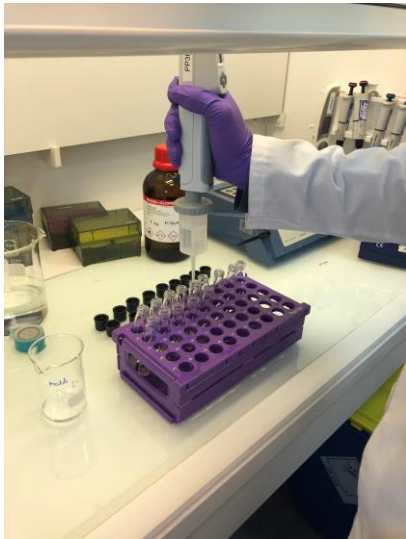
# Beta-Glucan

## Objective:

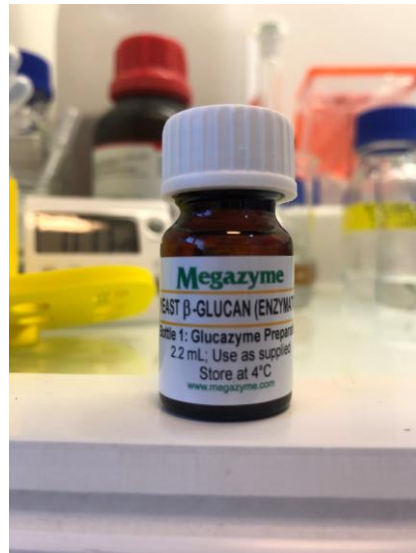
Determination of the beta-glucan content

Principle of the method: enzymatic hydrolysis of beta-glucan and dosage of glucose by HPLC

## Key steps of the method:



Add 0.4mL of KOH 2M, vortex and place in ice bath for 30 min  
Vortex every 10 min



Add 1.6 mL of acetate buffer and vortex  
Add 40µl de **Gluczyme** and mix slowly



Incubate at 40°C for 16 hours  
Add 8 ml of water and analyze by HPLC





## Comparison with codex protocol for beta-glucan determination

	Lesaffre Protocol	Food Chemical Codex Protocol
Solubilization	KOH 2M, vortex and place in ice bath for 30 min	KOH 2M, vortex and place in ice bath for 20 min
Hydrolysis	1 enzymatic digestion with enzyme mix: exo-1,3- $\beta$ -glucanase, endo-1,3- $\beta$ -glucanase, $\beta$ -glucosidase and chitinase  <i>(Glucazyme</i> acetate buffer 1.2M pH3.8 incubation at 40°C for 16 hours)	3 successive enzymatic digestions : <ul style="list-style-type: none"><li>• Lyticase (zymolyase) hydrolyzes poly-<math>\beta</math>(1<math>\rightarrow</math>3)-glucose 50°C 16h</li><li>• (1,6)-glucanase 80°C 15 min</li><li>• Mix of exo-beta-glucanase and betaglucosidase 40°C 1h</li></ul>
Detection	Detection of glucose by HPLC	Detection of glucose: oxidation with glucose determination reagent and spectrophotometer



**Jiri Kratochvil**  
**Lesaffre Yeast Corporation**  
**[j.kratochvil@lesaffre.com](mailto:j.kratochvil@lesaffre.com)**  
**Tel. 414-405-0215**