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Determination of β 1-6 linked β 1-3 glucans in the yeast cell walls (Wolff Method)

1 . Principle

The YCW is first submitted to hydrolysis with NaOH. The insoluble part is hydrolyzed by H2SO4 and, after neutralization, the glucose content is determined by an enzymatic kit (Roche)

2 . Material

- 50 ml capped flask
- lab centrifuge
- centrifuge tube
- pH - meter
- filter GF/C Whatmann
- 500 ml flask
- oven
- NaOH 0,1 N
- NaOH 32 %
- H2SO4 72 %
- Enzymatic kit for glucose determination (Boehringer/Roche) ref : 716251

3 . Method

In a 50 ml capped flask

♦ Weigh 2 g of YCW in a 50 ml capped flask.

♦ note the exact weight W5 and calculate the ratio RW5 =1/W5 for result in § 4

♦ Add 20 ml of 0,1 N, NaOH.

♦ Stir with magnetic stirring overnight at room temperature

♦ Put the solution into a centrifuge tube.

♦ Centrifuge 10 min

♦ Remove the supernatant and disperse the pellet in 40 ml of water

♦ Repeat 3 time this centrifuge operation

♦ Remove the supernatant after the last centrifuge

♦ In the centrifuge tube containing the pellet, add 25 ml of H2SO4 36 N.

♦ → Don't add the 25 ml of acid in one time. Add the acid slowly (5 ml by 5 ml) and stir the tube after each addition.

♦ Stir during 30 min at room temperature

♦ Put the entire solution into a capped flask and add 300 ml of water

♦ Close the flask and leave at 100°c during 6 H in a oven

♦ After cooling, neutralize at pH 4,5 with NaOH.

♦ Complete the solution at 500 ml.

♦ Filter with Wathman GFA membrane

♦ Determine the glucose in the filtrate with enzymatic kit (Roche) (see § 3.3.)