Determination of Beta-Glucan from Baker's Yeast—HPLC

SCOPE

This method is applicable to the quantification of (1-3), (1-6) beta glucan found in baker's yeast *(Saccharomyces cervisiae)* in raw materials. This method is appropriate for both the soluble and dispersible (not soluble) forms. This assay is equivalent to adopting the Chinese trade standard QB/T 4572-2013.

PRINCIPLE

Samples and Beta-glucan chemical reference substance are hydrolyzed with hydrochloric acid to free glucose. The free glucose is then quantified by HPLC.

Note: When yeast beta-glucan is hydrolyzed with hydrochloric acid, beta-glucan may not be completely hydrolyzed, or glucose hydrolyzed from beta-glucan may be destroyed when overheated, all this makes the result a little low to the fact. The error caused by the hydrolysis process should be corrected by beta-glucan chemical reference substance.

APPARATUS

- HPLC with Refractive Index Detector（RID） and Sugar column (6.5mm×300mm waters sugar pak-l or equivalent chromatographic column)
- Analytical balances, accurate to at least 0.001g
- Water bath, 30℃
- Vortex mixer
- Electric stove, 98-100℃
- Autoclave, 121℃
- pH meter

REAGENTS

- Ultrapure water
- Hydrochloric acid, 37%
- Beta-glucan chemical reference substance: Sigma C7821 or equivalent reagents
- Glucose solution(2g/L): Weigh 0.2g glucose accurately(accurate to 0.001g) after 98-100℃ of drying for 2h, and quantitatively on 100mL volumetric flask.

Sample Preparation

Weigh 100mg samples accurately(The figure is accurate to 0.001g), add to a 20mL glass nut tube, and add 6 mL hydrochloric acid(37%), mix well with vortex mixer. Put the tube into 30 ℃ water bath for 45min, every 15min mix once. Then put the solution into a 200mL Duchenne bottle, wash the tube twice with ultrapure water(about 100-120mL). Put the wash water into the Duchenne bottle. Put the bottle into the autoclave, treat for 121 ℃ and 60min. After cooling, adjust the pH of the solution to 6.0-7.0. Set the solution volume at 200mL. The solution was filtered though with 0.45μm membrane and determined by HPLC.

Weigh 100mg Beta-glucan chemical reference substance accurately(The figure is accurate to 0.001g), Process in the same way

Chromatographic Conditions

- chromatographic column: 6.5mm×300mm waters sugar pak-l or equivalent chromatographic column
- mobile phase: pure water
- flow: 0.6mL/min
- column temperature: 80℃
- injection volume: 20uL

HPLC Determination

According to the HPLC operating condition, the Beta-glucan chemical reference substance solution and the sample solution were determined. Quantitative analysis of the glucan in sample solution was done using standard curve method.

Standard curve

Absorb 1、2、3、4、5mL glucose solution separately to a 10mL volumetric flask, constant the volume. They are glucose standard solutions, which concentrations are 200、400、600、800、1000mg/L. Inject the glucose standard solutions with the above chromatographic conditions, and draw standard curve with chromatographic peak areas and concentrations

Calculation and expression of result

Calculation the content of beta-glucan in the test sample by HPLC data processor or according to the formula(1) .

$$X = \frac{A_1 \times 0.2 \times 100}{m_1 \times 1000} \times 0.9 \times F \quad \text{..... (1)}$$

X—Glucan content，%;

A_1—Glucose content by peak area of sample according to the standard curve，mg/L;

m_1—Weight of sample，g；

F—The empirical compensation coefficient for the loss of beta-glucan hydrolyzed to free glucose, calculate according to the formula(2);

0.2—The final volume of the sample solution, L；

0.9—Coefficient of converting glucose to glucan；

$$F = \frac{P \times (100 - W)}{(A_2 \times 0.2 \times 100)/(m_2 \times 1000) \times 0.9} \quad \text{..... (2)}$$

P—The content of Beta-glucan chemical reference substance (according to the test report provided by the reagent manufacturer)，%；

W—Water content of Beta-glucan chemical reference substance (according to the test report provided by the reagent manufacturer)，%；

A_2—Glucose content by peak area of Beta-glucan chemical reference substance according to the standard curve，mg/L；

m_2—Weight of Beta-glucan chemical reference substance, g;
Normally , the F value is about 1.25.