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Trehalose

海藻糖

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Foreword

This Standard was proposed by China Light Industry Council.

This Standard shall be under the jurisdiction of the Sub Technical Committee on Industrial Fermentation of National Technical Committee on Food Industry Standardization.

Drafting organization of this Standard: Nanning Sinozyme Biotechnology Co., Ltd., China National Research Institute of Food & Fermentation Industry.

Main drafters of this Standard: Hang Wei, Zhang Wei, Meng Zongjian, Guo Xinguang, Zhang Yunguang, Wei Yuqin.

Introduction

Trehalose was first-time extracted from *claviceps purpurea* of rye by Wigger's in 1832; and then put into mass production through fermentation yeast, extracting from maitake cells or enzymatic conversion of starch. China started industrial production in 2000. The sweetness of trehalose is equivalent to about 45% of that of sucrose. It has the characteristics of mild sweetness, refreshing sense of taste and no aftertaste after eating, as well as stability towards heat and acid. Because trehalose is non-reducing sugar, when existing with amino acids and protein, it does not produce browning (Maillard reaction) even being heated. The hygroscopicity of trehalose is low, so it remains dry even if the relative humidity reaches 95%. In addition, trehalose also has unique functions such as preventing starch from aging, preventing protein from denaturation and inhibiting lipid from oxidation. As a kind of excellent food ingredient, it can be directly consumed or used as food ingredient. Because of its special characteristics as non-reducing sugar formed by disaccharide molecules, a special protective film is formed under the harsh conditions such as high temperature, extreme cold and dehydration out of dry, which can effectively protect the bio-molecular structure from being destroyed, so it can be widely used in biologics, pharmaceuticals, cosmetics, agriculture and other industries.

Trehalose

1 Scope

This Standard specifies the product classifications, requirements, test methods, inspection rules, marks, packaging, transportation and storage of trehalose.

This Standard applies to the production, inspection and sales of the trehalose obtained through enzymatic conversion.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 191 Packaging - Pictorial marking for handling of goods (GB/T 191-2008, ISO 780: 1997, MOD)

GB 7718 General standard for the labeling of prepackaged foods

3 Terms and definitions

For the purposes of this Standard, the following terms and definitions apply.

3.1

Trehalose (α -D-glucopyranosyl- α -D-glucopyranoside)

The non-reducing disaccharide formed by two glucopyranose that are connected by a 1,1 glycosidic bond.

4 Chemical name, molecular formula, structural formula and relative molecular mass

Residue on ignition/%	≤	0.02	0.02	0.05
Loss on drying/%	≤	1.0	1.0	1.5
Chromaticity	≤	0.100	0.100	
Turbidity	≤	0.05	0.05	

6.3 Hygiene requirements

It shall comply with the relevant provisions of the State.

7 Test methods

7.1 Sensory test

TAKE an appropriate amount of samples; under the natural light, OBSERVE the color and shape of the sample visually; inspect whether there are impurities; TAKE a small amount of samples; TASTE them carefully (before tasting the second sample, it shall gargle with water); MAKE a record.

7.2 Content (High performance liquid chromatography)

7.2.1 Principle

Each component that comes into the chromatographic column at the same time - due to the difference of dissolution, adsorption, permeation and ion exchange between mobile phases and stationary phases - is allocated repeatedly for several times between two phases of the chromatographic column along with the mobile phases. Due to the moving speeds of each component in the chromatographic column are different, after passing through the chromatographic column of a certain length, the components are separated from each other, flow out the chromatographic column sequentially, and flow into the signal detector. INDICATE the spectral peak values of each component on the recorder or on the data processing device. QUANTIFY by normalization method or external standard method according to retention time.

7.2.2 Instruments

7.2.2.1 High performance liquid chromatograph (equipped with a differential detector).

7.2.2.2 Mobile phase degasser and 0.45μm microporous membrane.

7.2.2.3 Chromatographic column: amino column (4.6mm×300mm, 5μm).

7.2.2.4 Analytical balance: a sensitivity of 0.0001g.

7.2.2.5 Micro injector: 10 μ L.

7.2.3 Reagents

7.2.3.1 Water: secondary distilled water or ultrapure water.

7.2.3.2 Acetonitrile: chromatographically pure.

7.2.3.3 Trehalose standard products: purity $\geq$ 99.5%.

Note: If it takes crystalline trehalose as standard products, then the purity of anhydrous trehalose shall be multiplied by a conversion factor ($342.30 / 378.33 = 0.90$).

7.2.4 Analysis steps

7.2.4.1 Standard product preparation

The standard products need to be dried in a 60°C electro-thermal constant-temperature oven for 5h for weighing. WEIGH about 0.5g of trehalose standard products (7.2.3.3), accurate to 0.0001g; DISSOLVE with water (7.2.3.1) and dilute to 50mL; SHAKE well. FILTER with 0.45 μ m microporous membrane (7.2.2.2); COLLECT the filtrate for determination.

7.2.4.2 Sample preparation

The samples need to be dried in a 60°C electro-thermal constant-temperature oven for 5h for weighing. WEIGH about 0.5g of trehalose samples (7.2.3.3), accurate to 0.0001g; DISSOLVE with water (7.2.3.1) and dilute to 50mL; SHAKE well. FILTER with 0.45 μ m microporous membrane (7.2.2.2); COLLECT the filtrate for determination.

7.2.4.3 Determination of samples

The mobile phase is acetonitrile : water = 70 : 30. TURN on the differential detector before the day of determination, PREHEAT till stable, FIT on the column, INLET the mobile phase with a flow rate of 0.1mL / min, BALANCE overnight. Before formal injection analysis, INLET the mobile phase used TO the reference cell for more than 20min, RESTORE normal flow path, to make the mobile phase flow through the sample cell, ADJUST the flow rate to 1.0mL / min, ADJUST the baseline until the baseline is stable, INLET 10L of the standard solution (7.2.4.1) and the prepared sample (7.2.4.2) respectively. According to the retention time of standard products, determine the chemical composition of the sugar components in the sample. According to the peak area of the sample, calculate the percentage content of sugar components by external standard method.

7.2.4.4 Calculation results

WEIGH 30g of the sample, accurate to 0.1g; ADD water to dissolve and dilute to 100mL; SHAKE well. USE 1cm cuvette to take water as the blank control; DETERMINE the absorbance of the sample solution at the wavelength of 420nm and 720nm respectively; RECORD the readings.

7.6.3 Calculation results

7.6.3.1 Chromaticity

CALCULATE the chromaticity according to formula (4):

$$X_4 = OD_{420} - OD_{720} \dots\dots\dots (4)$$

Where:

X_4 - Colorimetric values of samples;

OD_{420} - Absorbance of sample solution at the wavelength of 420nm;

OD_{720} - Absorbance of sample solution at the wavelength of 720nm.

7.6.3.2 Turbidity

The turbidity value of samples is the absorbance of samples at the wavelength of 720nm.

7.6.4 Precision

The absolute difference between the two independent determination results obtained under the repeatability conditions shall not exceed 0.2% of the arithmetic mean.

8 Inspection rules

8.1 The products dried at a time are deemed as one batch. The maximum batch shall not exceed the output per shift.

8.2 Each batch of products shall exit factory after being inspected as qualified by the inspection department of the factory, and enclose with product quality certification.

8.3 Sampling methods

8.3.1 TAKE samples according to the specifications in Table 2 and Table 3.

8.4.2 Type inspection

8.4.2.1 Inspection items: all required items in this Standard.

8.4.2.2 Under normal circumstances, type inspection of the same type of products shall be performed at least once every six months. If there are one of the following circumstances, it shall be performed as well:

- a) When there are significant changes on raw and auxiliary materials;
- b) When key process or devices are changed;
- c) When the production of new products or normally produced products is resumed after shutting down for 3 months;
- d) When there are great difference between factory inspection results and last type inspection results;
- e) When National Quality Supervision and Inspection Agency requires inspection according to the relevant provisions.

8.5 Determination rules

8.5.1 If the test results of sensory or 1 ~ 2 physical and chemical indicators are unqualified, it can take doubled amount of samples from this batch of products, to re-inspect the unqualified items. If the re-inspection results indicate that one item is unqualified, this batch of products are determined unqualified.

8.5.2 If one of the hygiene indicators is unqualified, this batch of products are determined unqualified.

9 Marks, packaging, transportation and storage

9.1 Labeling for prepackaged products shall meet the requirements of GB 7718.

9.2 Pictorial marking for handling of goods shall comply with the specifications of GB/T 191.

The outer packages shall indicate product name, manufacturer name and address, net weight, production date, expiration date, executive standard number and quality levels.

9.3 The packages and containers shall be clean, sanitary, no damage.

9.4 During transportation, it shall prevent dust, flies, sun and rain. It is prohibited to be packaged and transported with toxic and hazardous substances.

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