Japan Customs Analysis Methods

No. 110

Analysis Method for Determination of the Alpha Conversion of Starch

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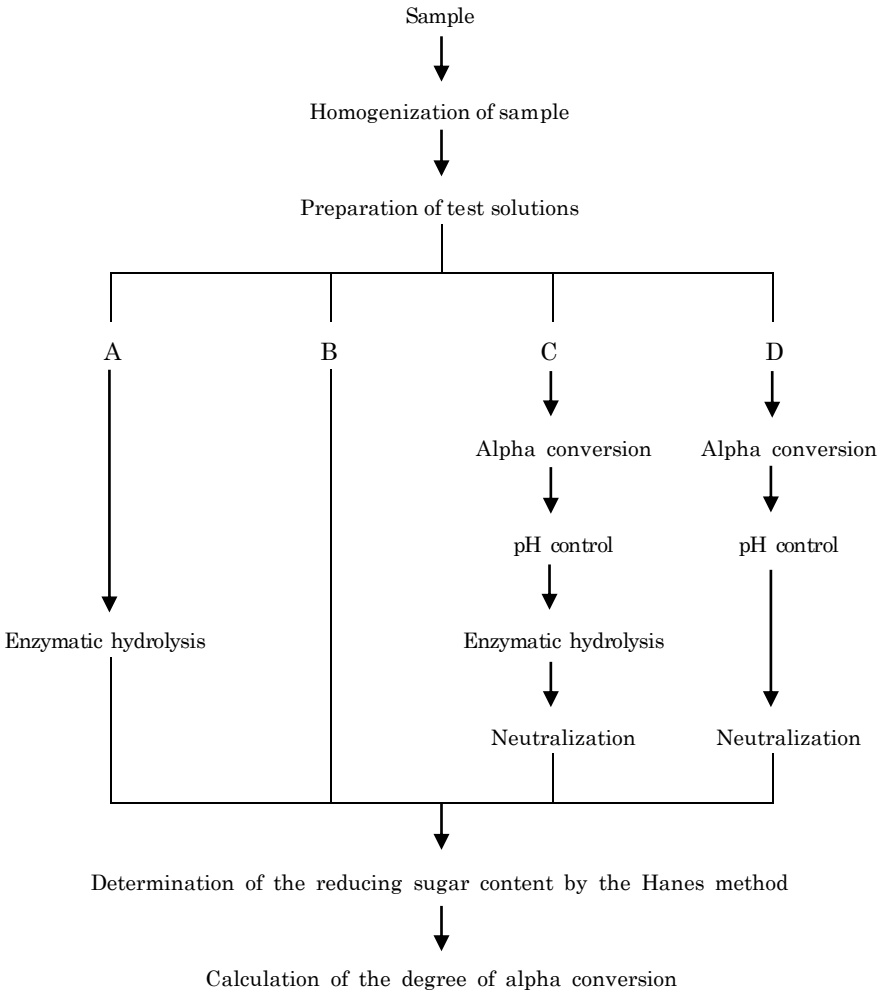
1. Scope

This analysis method is applied to gelatinized starch, cereal powder, boiled red beans or the like for which the degree of alpha conversion of starch is required.

2. Outline of Test Method

In this method, the analysis is carried out according to the following flow-diagram.



3. Reagents

(a) 2 mol/L sodium hydroxide aqueous solution

Dissolve 8 g of sodium hydroxide in water and dilute to 100 mL.

(b) 2 mol/L acetic acid aqueous solution

Dissolve 12 g of acetic acid in water and dilute to 100 mL.

(c) 0.2 M acetic acid buffer (pH4.8)

Add 120 mL of 0.2 mol/L sodium acetate aqueous solution to 80 mL of 0.2 mol/L acetic acid aqueous solution and then adjust it to pH4.8 by adding 0.2 mol/L sodium acetate aqueous solution or 0.2 mol/L acetic acid aqueous solution.

(d) Enzyme solution

Dissolve glucoamylase (1, 4 - α - D - Glucan glucohydrolase EC 3.2.1.3) in 0.2 M acetic acid buffer (pH4.8) so that the enzymatic activity becomes 20 units / mL.[1]

Note 1) One unit is defined as the quantity of enzyme which produces 10 mg of glucose for 30 min when incubated with 1% soluble starch as the substrate at pH4.5 and 40ºC.

(e) Reagents for the Hanes method

As for the reagents for the Hanes method , i.e. Hanes solutions A, B and C, 0.1 mol/L sodium thiosufate solution, 1% soluble starch solution and deproteinizing agent (Solutions A and B), refer to the Customs Analysis Method No. 108 "Method of Quantitative Analysis of Sucrose in Confectionery".

4. Sample Preparation

Analysis samples should be prepared in an appropriate manner according to their conditions.[2] Solid samples should be crushed with a grinder or the like. Pasty or wet samples (for frozen red beans, the skins should previously be removed after defrosting) should be homogenized in a mortar. The sample preparations above must be performed without heating.

Accurately weigh about 0.6 g[3] of analysis sample in a beaker. Add water to the beaker and transfer a part of the sample liquid into a glass homogenizer. Grind the sample liquid while cooling with ice. After grinding, transfer the entire contents into a 100 mL volumetric flask. Repeat this procedure until the entire quantity of the analysis sample is treated and transferred to the flask, and then dilute to volume with water.

Note 2) In cases where the sample contains a relatively large amount of fat, remove fat according to the following procedure. After crushing the sample, put about 20 g of it into a beaker, add 200 mL of diethyl ether and stir thoroughly. Thereafter, filter it to remove ether. Transfer the sample into another beaker, add 200 mL of ethyl alcohol, stir thoroughly and filter it. Put the washed sample in a desiccator and remove the remaining ethyl alcohol by using an aspirator to depressurize.

Note 3) According to the amounts of ingredients other than starch, such as moisture, adjust the sampling amount so that the sample contains about 0.5 g of starch.

5. Hydrolysis Procedure

Mark four 30 mL-Erlenmeyer flasks "A", "B", "C" and "D" respectively. Put 10 mL of the suspension liquid prepared in 4. (mix and homogenize the liquid just before attempting to dispense[4]) into each of the flasks using a whole pipette. Put the flasks in a constant-temperature shaker water bath which has previously been set at 37°C.

Add 2 mL of 2 mol/L sodium hydroxide aqueous solution to flasks "C" and "D" using a whole pipette in conditions where the liquids are suspended and homogenized[5] and then gelatinize the liquids by keeping the flasks in the constant temperature shaker water bath.

Wait for 30 min and add 3 mL of 2 mol/L acetic acid aqueous solution to flasks "C" and "D" using a whole pipette for neutralization. Add 2 mL of the enzyme solution to flasks "A" and "C" and keep them in the constant temperature shaker water bath for two hours for enzymatic reaction.

After the enzymatic reaction, add 1 mL of 2 mol/L hydroxide solution to flasks "C" and "D" using a whole pipette for neutralization. Upon completion of the reaction, transfer the entire volume of the test solutions in the flasks "A", "B", "C" and "D" into four separate 200 mL volumetric flasks which have previously been marked as "A", "B", "C" and "D". Furthermore, add to

each of the flasks 10 mL each of deproteinizing agent solutions A and B in order, and dilute them to volume with water.

Filter these solutions through a paper filter respectively and use filtrates as test solutions "A," "B," "C" and "D".

Note 4) Suspended and homogenized liquid must be dispensed for example by collecting the liquid quickly while mixing with a stirrer.

Note 5) Do not add 2 mol/L hydroxide solution to test solutions in conditions where there are sediments at the bottom of the flasks.

6. Titration of Reducing Sugar by the Hanes Method

Dispense 5 mL each of the test solutions (A, B, C and D) prepared in 4. into large test tubes (2.5 – 3 cm in diameter) using whole pipettes, respectively. Put 5 mL of water as a control into another large test tube.

Accurately add 5.0 mL of Hanes solution A to each of the test tubes and mix well. Place them in a boiling water bath and heat precisely for 15 min.

Immediately upon finishing the heating, cool them under running water.

Subsequently, add 5 mL of Hanes solution B to each of the test tubes and mix thoroughly. After adding 3 mL of Hanes solution C and mixing further, immediately titrate[6, 7] them with 0.01 mol/L sodium thiosulfate solution (to be made by diluting 0.1 mol/L sodium thiosulfate solution to ten times as much at the time of use).

During the titration, the test tubes should be shaken to mix well. Add 3 to 4 drops of 1% soluble starch solution when the titrating solution changes color to pale yellowish white, and then continue titrating carefully until the end-point, at which a purple color disappears. Read the titration volume of 0.01 mol/L sodium thiosulfate solution.

Follow the same steps for the control and read the titration volume of 0.01 mol/L sodium thiosulfate solution.

Note 6) The operations from the addition of Hanes solution A to the titration must be done immediately. In particular, after adding Hanes solution B, the remaining steps (until the titration is finished) must be done immediately on a tube-by-tube basis.

Note 7) It is desirable to conduct multiple titrations for a single test solution using a small volume burette.

7. Calculation of the Degree of Alpha Conversion

Obtain the consumed amount of the sodium thiosulfate for each test solutions by subtracting the titration volume (mL) of the test solutions (A, B, C and D) from that (mL) for the control. Calculate the degree of alpha conversion (%)[8] by the following formula. Round off fractions to the first decimal place.

$$\text{The degree of alpha conversion} = \frac{a - b}{c - d} \times 100$$

Where —

a: consumed amount of the sodium thiosulfate solution for test solution A (mL)

b: consumed amount of the sodium thiosulfate solution for test solution B (mL)

c: consumed amount of the sodium thiosulfate solution for test solution C (mL)

d: consumed amount of the sodium thiosulfate solution for test solution D (mL)

Note 8) In case of samples containing dextrin, the apparent values of the degrees of alpha conversion will become higher due to the hydrolysis of the dextrin with the enzyme used.

8. References

(1) The Pharmaceutical Society of Japan, "Standard methods of analysis for hygienic chemists: with commentary, Methods of analysis in health science" (in Japanese), Kanehara-Shuppan (2010).

(2) Customs Analytical Method No. 108, "Method of Quantitative Analysis of Sucrose in Confectionery" (Original, in Japanese; updated in 2007).

(3) Ikeda H., Gunji M., Takayama N., Tomita K. (2002), Reports of the Central Customs Laboratory **41**:41 (in Japanese).

(4) Sekikawa Y., Kato T. (1985), Reports of the Central Customs Laboratory **25**:25 (in Japanese).

(5) Nikoku J., "Handbook of Starch Science" (in Japanese), Asakura Publishing (1977).