Japan Customs Analysis Methods

No. 114

Quantitative Analysis of Reducing Sugars in Sugar Preparations consisting of Sugar and Dextrin

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1. Scope

This analysis method is applied to sugar preparations which consist of sugar and dextrin and which require the determination of their "reducing sugar contents, expressed as dextrose on dry substances," as referred to in Note 2 to Chapter 35 in Customs Tariff Law (Appendix Table–Customs Tariff Schedule).

2. Outline of Test Method

This analytical method is applied for products containing sucrose and dextrin (starch degradation products) to determine reducing sugars, e.g. dextrose and maltose, contained in the dextrin. The procedure is summarized below.

(1) Determination of moisture content

(2) Determination of direct reducing sugars by the Lane-Eynon method

(3) Determination of sucrose by the Lane-Eynon method

(4) Determination of the content of dextrin

(5) Calculation of DE value

3. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

3.1. Preparation of standard dextrose solution for making a calibration curve

Accurately weigh about 1 g of dextrose, transfer to a 1,000 mL volumetric flask, and dilute to volume with water.

3.2. Preparation of standard dextrose solution for standard-addition

Accurately weigh about 4 g of dextrose, transfer to a 100 mL volumetric flask, and dilute to volume with water.

3.3. Preparation of standard invert sugar solution and other reagents

(1) Standard invert sugar solution

Accurately weigh 4.75 g of sucrose, transfer with 90 mL of water to a 500 mL volumetric flask, and add 5 mL of hydrochloric acid (specific gravity, 1.18). After leaving to stand at 20–30°C for three days, dilute the solution to volume with water and store in a cool dark place.

Transfer a 50 mL portion of the solution above to a 200 mL volumetric flask, neutralize with 1 mol/L sodium hydroxide aqueous solution using phenolphthalein as an indicator, and dilute to volume with water.

Use the solution as standard invert sugar solution for the standardization of Fehling's Solution.

(2) 1% Methylene Blue solution

Dissolve 1 g of methylene blue in water to make 100 mL.

(3) Fehling's Solution

Solution A: Dissolve 34.639 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water to make

exactly 500 mL, leave it for two days, and then filter.

Solution B: Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 50g of sodium hydroxide in water to make exactly 500 mL, leave it for two days, and then filter.

3.4. Standardization of Fehling's Solution

Put 5.0 mL of Fehring's Solution A and 5 mL of Fehring's Solution B into a 200 mL Erlenmeyer flask containing a few glass beads and add from a 50 mL burette 19.5 mL of the standard invert sugar solution. After boiling it on an electric stove (heater) for two minutes, add four drops of the methylene blue solution. Complete titration within a total boiling time of three minutes by dropwise addition of the standard invert sugar solution—without preventing boiling—until the blue color disappears. Repeat titration twice and calculate the mean of three parallel titrations. (1)

Obtain the *factor* of the Fehring's Solution from the following formula:

$$Factor^{(2)} = 20.36 / A$$

Where– A: Volume (mL) of the standard invert sugar solution required

Note 1) Use the mean value of the three parallel titrations as "A"; duplicate titrations must agree to within 0.1 mL in the volume of the sugar solution required

Note 2) Calculate *factor* by rounding off fractions to the third decimal place; the *factor* must be within a range of 1±0.02.

3.5. Preparation of buffers and enzyme solutions

3.5.1. 0.1M acetic acid buffer (pH 4.6)

Weigh 6 g of glacial acetic acid in a beaker, dissolve in 100 mL of water and adjust the pH to 4.6 using a 5% sodium hydroxide aqueous solution. Transfer the solution to a 1,000 mL volumetric flask and dilute to volume with water.

3.5.2. 0.2M acetic acid buffer (pH 4.8)

By adding 120 mL of a 0.2M sodium acetate to 80 mL of 0.2M acetic acid, adjust the pH of the mixed solution to 4.8.

3.5.3. Invertase solution

Dissolve invertase in the 0.1M acetic acid buffer (pH 4.6) so that the concentration of invertase becomes 400 units/mL.

3.5.4. Enzyme solution for determination of dextrin

Dissolve glucoamylase (1,4-α-D-Glucan glucohydrolase EC 3.2.1.3) and α-amylase (α-1,4-Glucan 4-glucanohydrolase EC 3.2.1.1) in the 0.2M acetic acid buffer (pH 4.8) so that their concentrations become 20 units/mL(3) and 80 units/mL,(4) respectively. (5)

Note 3) One unit represents the amount of enzymes able to produce 10 mg of glucose from soluble starch (as substrate) every 30 minutes at 40°C and pH 4.5.

Note 4) One unit represents the amount of enzymes able to produce 0.18 mg of reducing sugar, expressed as glucose, from soluble starch (as substrate) per minute at 40°C and pH 6.0.

Note 5) When using enzymes whose potencies or units have been determined based on different definitions, confirm in advance that their recovery rates in digesting 50 mg of corn starch are 100%.

3.5.5. Glucose determination kit

Use a commercially available enzyme-based assay kit for the determination of glucose (dextrose).

3.6. Deproteinizing agent

Solution A: Dissolve 2 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) in 100 mL of water.

Solution B: Dissolve 1.8 g of barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$] in 100 mL of water.

4. Preparation of samples

Prepare and collect analysis samples in appropriate manners, e.g. sample reduction methods, etc., depending on their conditions presented. For powder or crystal mixtures, grind them with a grinder or a mixer. For pasty or wet materials, homogenize them by mixing in mortars. In any case, collect relatively large amounts of samples randomly, and grind or mix them to uniformity.

5. Procedure

5.1. Determination of moisture content

Accurately weigh about 2 g of the sample homogenized in 4. in a weighing bottle which has been previously dried to a constant weight. Dry it in a vacuum oven at a temperature of 70–75°C for four hours, cool to room temperature in a desiccator and weigh. Repeat vacuum drying until the loss in weight does not exceed 2 mg per hour in the drying period.

Calculate the moisture content in the sample from the following formula. Round off fractions to the first decimal place.

$$\%, \text{moisture content} = \{(W_0 - W_1) / W_0\} \times 100$$

Where–

W_0: Amount (g) of sample collected

W_1: Weight (g) of sample after drying

5.2. Preparation of sample solution

Accurately weigh 15 g of the sample homogenized in 4., and dissolve in water. Transfer the solution to a 500 mL volumetric flask and dilute to volume with water.

5.3. Determination of Direct Reducing Sugar in Sample

5.3.1. Preparation of test solution

Put a 100 mL portion of the sample solution prepared in 5.2. in a 200 mL volumetric flask, add 10 mL of the standard dextrose solution for standard-addition prepared in 3.2., and dilute to volume with water.

5.3.2. Preparation of blank solution

Put a 10 mL portion of the standard dextrose solution for the standard-addition prepared in 3.2 in a 200 mL volumetric flask, add about 2.5 g[(6)] of sucrose and dissolve by adding a small amount of water. Dilute the solution to volume with water.

Note 6) This is a reference on the amount of sucrose added specifically for samples consisting of *83 % of sucrose* and *17 % of dextrin*. Thus, when analyzing samples with different compositions, change the amount of sucrose added so that the sucrose concentrations in the test solution and the blank solution become almost the same.

5.3.3. Titration

Put 5.0 mL of Fehring's Solution A and 5 mL of Fehring's Solution B into a 200 mL Erlenmeyer flask. Add from a 50 mL burette 15 mL of the test solution prepared in 5.3.1., and titrate as described in 3.4 (preliminary titration).

Then, put 5.0 mL of Fehring's Solution A and 5 mL of Fehring's Solution B into another 200 mL Erlenmeyer flask. Add from a 50 mL burette the test solution within 1 mL of the anticipated end point from the result of the preliminary titration above, and titrate in the same manner as in the preliminary titration.

Multiply by the *factor* of the Fehling's Solution the volume (mL) of the test solution required in order to obtain the corrected titre, X (mL). Obtain the concentration of the direct reducing sugars, Ds (mg/100mL) from the titre, X (mL), by reference to the appended Lane-Eynon's Table (dextrose). Similarly, perform titration with the blank solution and obtain the concentration of the direct reducing sugars, Ds' (mg/100mL), from the corrected titre, X' (mL), by making reference to the appended Lane-Eynon Table (dextrose).

Using Ds and Ds', calculate the content (%) of direct reducing sugar from the following formula:

$$\%, \text{direct reducing sugar} = \frac{(Ds - Ds')}{S} \times \frac{500}{100} \times \frac{200}{100} \times 100$$

Where–

Ds: Concentration (mg/100mL) of direct reducing sugar in test solution, obtained by reference to the appended Lane-Eynon Table (dextrose).

Ds': Concentration (mg/100mL) of direct reducing sugar in blank solution, obtained by reference to the appended Lane-Eynon's Table (dextrose).

S: Weight (mg) of sample collected in 5.2.

$$\text{(Ref.) Dilution rate} = \frac{500}{100} \times \frac{200}{100}$$

5.4. Determination of sucrose in sample

5.4.1. Inversion reaction

Put a 20 mL portion of the sample solution prepared in 5.2. into a 200 mL volumetric flask and add 1 mL of the invertase solution. Hydrolyze the sample solution by placing the flask in a water bath at a constant temperature of 37°C for 30 minutes, add 5 mL each of the deproteinizing solutions A and B, and mix thoroughly. Dilute the deproteinized solution to volume with water and filter. Use the filtrate as test solution for the determination of sucrose.

5.4.2. Titration and calculation of sucrose content

Titrate with the test solution prepared in 5.4.1. in accordance with the procedure in 5.3.3. Obtain the concentration of the invert sugar, T (mg/100mL), by reference to the appended Lane-Eynon's Table [invert sugar (without sucrose)] and calculate the invert sugar content (%) from the following formula.

$$\%, \text{invert sugar} = \frac{T}{S} \times \frac{500}{20} \times \frac{200}{100} \times 100$$

Where–

T: Concentration (mg/100mL) of invert sugar in test solution, obtained by reference to the appended Lane-Eynon's Table [invert sugar (without sucrose)].

S: Weight (mg) of sample collected in 5.2.

Next, from the titres (X and X') obtained in 5.3.3, obtain the contents of the direct reducing sugar in the sample and blank solutions, DRs (mg/100mL) and DRs' (mg/100mL), by reference to the appended Lane-Eynon Table [Invert sugar (without sucrose)]. Calculate from the following formula the content of the direct reducing sugar, expressed as invert sugar, in dextrin contained in the sample, A (%).[7]

$$\text{A (\%)} = \frac{(\text{DRs} - \text{DRs}')}{S} \times \frac{500}{100} \times \frac{200}{100} \times 100$$

Where–

DRs: Concentration (mg/100mL) of direct reducing sugar in test solution, obtained by reference to the appended Lane-Eynon Table [Invert sugar (without sucrose)].

DRs': Concentration (mg/100mL) of direct reducing sugar in blank solution, obtained by reference to the appended Lane-Eynon's Table [Invert sugar (without sucrose)].

S: Weight (mg) of sample collected in 5.2.

$$\%, \text{Sucrose content} = \{\text{invert sugar content (\%)} - \text{A (\%)}\} \times 0.95$$

Note 7) The reason why two types of direct reducing sugar contents in dextrin are calculated is that the content of direct reducing sugar, expressed as invert sugar, is needed for the determination of sucrose content, whereas that expressed as dextrose is needed for the determination of DE value.

5.5. Determination of dextrin content

5.5.1. Enzymatic digestion

Put a 2 mL portion of the sample solution prepared in 5.2. in a 100 mL volumetric flask and add 5 mL of the enzyme solution for determination of dextrin. Place the flask in a water bath at a constant temperature of 37°C for two hours for enzymatic digestion, add 5 mL each of the deproteinizing solutions A and B, and mix thoroughly. Dilute the solution to volume with water and filter. Use the filtrate as test solution for the determination of dextrin.

5.5.2. Preparation of calibration curve for dextrose

Put 5, 10, 15, 20 and 25 mL of the standard dextrose solutions prepared in 3.1. in 100 mL volumetric flasks, respectively, and dilute the solutions to volume with water. Use them as standard solutions for constructing a calibration curve.

Using a commercially available dextrose determination kit, e.g. glucoxidase-peroxidase-based assay kits, described in 3.5.5., prepare a calibration curve by plotting absorbance against dextrose concentration (mg/ml) for each of the standard solutions

prepared above. Construct the calibration curve simultaneously during the procedure in 5.5.3.

5.5.3. Determination of dextrose and calculation of dextrin content

Utilizing the same assay kit described in 3.5.5., quantify dextrose in the test solution prepared in 5.5.1. and determine the concentration (mg/ml) of dextrose in the test solution using the calibration curve constructed in 5.5.2. Calculate the dextrin content (%) in the sample from the following formula.[8, 9]

$$\%, \text{dextrin} = \frac{\text{Dextrose}\,(\%) \times 0.9 \times \text{Dilution rate}}{\text{S}} \times 100$$

Where–

S: Amount (mg) of sample collected in 5.2.

Note 8) In the calculation, the dextrin content is deemed to be equal to "dextrose (%) × 0.9."

Note 9) Dextrin content may be calculated as a balance. In that case, calculate the dextrin content from the following formula:

Dextrin content (%) = 100 – {sucrose content (%) +moisture content (%)}

However, when the calculated value looks doubtful due to the differences from those in the attachments and composition table provided, determine the exact value with assay.

5.6. Calculation of DE

Calculate the content of reducing sugars, expressed as dextrose on the dry substance, in dextrin contained in the sample by applying the following formula with the values obtained in 5.3.3. and 5.5.3. Round off fractions to the first decimal place.

$$\text{DE} = \frac{\text{Direct reducing sugar content}\,(\%)\,\text{in dextrin}}{\text{Dextrin content}\,(\%)\,\text{in sample}} \times 100$$

Where–

DE: Content (%) of reducing sugar, expressed as dextrose on the dry substance, in the dextrin contained in the test sample.

6. References

(1) 中村道徳, 貝沼圭二 「澱粉･関連糖質実験法」学術出版センター (1986)

(2) 三国二郎 監修 「澱粉化学ハンドブック」 朝倉書店 (1977)

Appendix

Lane-Eynon Table (Invert sugar and Dextrose)

mL sugar solution required	Saccharides	
	Invert sugar (without sucrose) mg/100ml	Dextrose (anhydrous) mg/100ml
15	336	327
16	316	307
17	298	289
18	282	274
19	267	260
20	254.5	247.4
21	242.9	235.8
22	231.8	225.5
23	222.2	216.1
24	213.3	207.4
25	204.8	199.3
26	197.4	191.8
27	190.4	184.9
28	183.7	178.5
29	177.6	172.5
30	171.7	167
31	166.3	161.8
32	161.2	156.9
33	156.6	152.4
34	152.2	148
35	147.9	143.9
36	143.9	140
37	140.2	136.4
38	136.6	132.9
39	133.3	129.6
40	130.1	126.5
41	127.1	123.6
42	124.2	120.8
43	121.4	118.1
44	118.7	115.5
45	116.1	113
46	113.7	110.6
47	111.4	108.4
48	109.2	106.2
49	107.1	104.1
50	105.1	102.2