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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 22031-2008

Replacing GB/T 22031-2008

**National food safety standard -- Determination of added citrate
content in cheese and processed cheese products**

干酪及加工干酪制品中添加的柠檬酸盐含量的测定 酶-比色法

(English Translation)

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Table of Contents

Foreword
1 Scope
2 Normative references
5 Expression analysis
6 Precision

Foreword

This standard replaces GB/T 22031-2008 "cheese and processed cheese products of added citrate content determination of enzyme - colorimetric method." the Standard Appendix A, Appendix B normative appendix.

This standard replaces the standards previously issued as follows.

--GB/T 22031-2008.

National Food Safety Standard Determination of cheese and processed cheese products added citrate

1 Scope

This standard specifies the cheese and processed cheese products of added citrate content (citric acid meter) measurement method.

This standard applies to the determination of citrate content in cheese and processed cheese products added to.

2 Normative references

The standard file referenced in the application of this standard is essential. For dated references, only the edition date of the note Apply to this standard. For undated references, the latest edition (including any amendments) applies to this standard.

Principle 3

Determination of the total sample of citrate, deducting the amount of raw materials into the sample citrate (0.04 times the lactose conversion), namely Samples of added citrate content. Citrate, citric acid count.

Step 4 Analysis

Determination of the total content of the samples and citrate calculated according to Appendix A of the regulations.

Determination and lactose content in the sample is calculated in accordance with Appendix B of the regulations.

5 Expression analysis

Samples at levels of added citrate (1) is calculated.

$$w_a = w_c - r w_i \quad (1)$$

Where.

w_a -- sample added citrate content in terms of citric acid, the mass fraction (%) represented;

w_c -- total content of citric acid in the sample, in units of mass fraction (%);

w_i - lactose content in the sample, in units of mass fraction (%);

r - the ratio of raw milk or whey and lactose content of citric acid content (citric acid/lactose) ($r = 0.04$).

The arithmetic mean of two under the same condition of independent determination results indicated that the results of reservations to two decimal places.

6 Precision

Two independent determination results under the absolute difference in repeatability condition must not exceed 10% of the arithmetic mean.

Appendix A

(Normative)

Cheese and processed cheese products of enzymatic assay of citrate content A.1 Scope this standard applies to enzymatic assay cheese and processed cheese products citrate content.

A.2 Principle

Citrate lyase (CL) will citrate into oxaloacetate and acetate, malate dehydrogenase (MDH) and lactate dehydrogenase Catalase (LDH) catalyzed decarboxylation of oxaloacetate and decarboxylation of malonic at reduced nicotinamide adenine dinucleotide (of NADH) in the presence of Keto acid salt, were transformed into L- L- malic acid and lactic acid. NADH is oxidized in the reaction it became NAD. Determination of the sample solution at 340 nm in NADH absorbance difference calculation citrate content in the sample.

A.3 Reagents and materials Unless otherwise specified, the reagents used in this method are analytically pure water GB/T 6682 provisions of secondary water.

A.3.1 trichloroacetic acid solution (200 g/L). Weigh 20 g of trichloroacetic acid (CCl_3COOH) was dissolved in water, mix, and set the volume to 100 mL.

A.3.2 sodium hydroxide solution (200 g/L). Weigh 20 g of sodium hydroxide (NaOH), dissolved in water transferred to 100 mL volumetric flask, Volume. Mixed thoroughly.

A.3.3 sodium hydroxide solution (40 g/L). Weigh 4 g of sodium hydroxide (NaOH), dissolved in water transferred to 100 mL volumetric flask, Yung. Mixed thoroughly.

A.3.4 sodium hydroxide solution (4 g/L). Weigh 0.4 g of sodium hydroxide (NaOH), dissolved in water transferred to 100 mL volumetric flask, Yung. Mixed thoroughly.

A.3.5 zinc chloride solution (0.8 g/L). Weigh 0.8 g of zinc chloride (ZnCl_2), dissolved in water transferred to 1000 mL volumetric flask, constant volume.

Mixed thoroughly.

A.3.6 buffer (pH 7.8). Weigh 7.13 g glycylglycine ($\text{H}_2\text{NCH}_2\text{CONHCH}_2\text{CO}_2\text{H}$), was dissolved in 70 mL of water, transferred to a 100 mL volumetric flask. Adjusted with sodium hydroxide solution (A.3.2) pH 7.8, was added 10 mL of zinc chloride solution (A.3.5) and dilute to 100 mL.

Mixed thoroughly. Stored in a refrigerator $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$, the solution can save around.

A.3.7 sodium bicarbonate solution (4.0 g/L). Weigh 4.0 g of sodium bicarbonate (NaHCO_3), dissolved in water transferred to 1000 mL volumetric flask, Volume. Mixed thoroughly.

A.3.8 nicotinamide adenine dinucleotide (NADH) solution. take 50 mg of nicotinamide adenine dinucleotide disodium salt ($\text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_{14}\text{P}_2\text{Na}_2$) and 100 mg of sodium bicarbonate (NaHCO_3), was dissolved in 10 mL of water.

A.3.9 ammonium sulfate solution (422 g/L). Weigh 42.2 g of ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$], dissolved in water transferred to 100 mL volumetric flask, Yung. Mixed thoroughly.

A.3.10 malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) suspension. ammonium sulfate solution (A.3.9) are dissolved Apple Dehydrogenase (pig heart, EC 1.1.1.37) and lactate dehydrogenase (rabbit, EC 1.1.1.27), so malate dehydrogenase (MDH) activity not less than 600 IU/mL and lactate dehydrogenase activity is not less than 1400 IU/mL. Slowly stir into suspension after storage at $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ refrigerator and the solution can be kept for one year.

A.3.11 citrate lyase (CL) solution. $0\text{ }^{\circ}\text{C}$ dissolved in water with citrate lyase (*Enterobacter aerogenes*, EC 4.1.3.6), make lemon Citric acid lyase activity of not less than 40 IU/mL. Slowly stir into suspension after storage at $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$ refrigerator, this solution can be Paul Deposit one week; at $-20\text{ }^{\circ}\text{C}$ refrigerator, you can save around.

A.3.12 citric acid standard solution (160 $\mu\text{g/mL}$). Weigh 175 mg of citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$), dissolved in water was transferred to a 1000 mL volumetric flask,

constant volume. Mixed thoroughly.

A.4 instruments and equipment A.4.1 Balance. a sense of the amount of 0.1 mg.

A.4.2 pH meter. accuracy of 0.1.

A.4.3 grinder.

A.4.4 stoppered graduated colorimetric tube. 10 mL, dividing the value of 0.1 mL.

A.4.5 medium speed filter paper.

A.4.6 UV-Vis. 340 nm, 1 cm cuvette.

A.4.7 water bath.

A.5 Sample preparation A.5.1 cheese Remove shell surface or moldy cheese, the sample representative. With a grinder (A.4.3) The sample was crushed, and mix.

A.5.2 cheese products Select a representative sample pulverized with a grinder (A.4.3), and mix.

A.6 analysis step

A.6.1 Preparation of test solution Weigh 1 g (accurate to 0.0001 g) sample (A.5), was dissolved in 50 mL of hot water (40 °C ~ 50 °C), the entire transferred into 100 mL Flask, cooled to 20 °C. Then add 10 mL of trichloroacetic acid solution (A.3.1), volume with water, mix well stand for 30 min. use Filter paper (A.4.5) filtered and filtrate was discarded early about 10 mL. Draw 25 mL filtrate in a beaker with sodium hydroxide solution (A.3.3) adjustment filter Solution to pH 4, and then sodium hydroxide solution (A.3.4,) to adjust the pH to 8. The beaker was transferred into a 100 mL volumetric flask, add water Yung. While doing the blank test.

A.6.2 Determination

A.6.2.1 Drawing standard curve Before using a reagent to room temperature. Imbibe 0.00 mL, 0.50 mL, 1.00 mL, 1.50 mL, 2.00 mL (equivalent to 0.80, 160,200,320μg citric acid) groups citric acid standard solution (A.3.12), were placed in 10 mL colorimetric tube (A.4.4), each added 1.00 mL buffer solution (A.3.6), 0.10 mL NADH solution (A.3.8), 0.02 mL malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) Suspension (A.3.10), shake. Holding 5 min at 20 °C ~ 25 °C water bath (A.4.7), the water volume to 5.00 mL. One group with a 1 cm cuvette (A.4.6), with air as reference, measured in each colorimetric tube solution absorbance at a wavelength of 340 nm at A0. Another plus for each group Into 0.02 mL of citrate lyase (A.3.11), mixing, holding 10 min at 20 °C ~ 25 °C water bath (A.4.7), and by 1 cm Cuvette (A.4.6), with air as reference, measured in each colorimetric tube solution absorbance at a wavelength of 340 nm at A10. According to (2) is calculated Absorbance A, citric acid content of the ordinate, the absorbance A is the abscissa, the standard curve.