National Food Safety Standard of P. R. China

GB 22031 - 2010

National Food Safety Standard

Determination of Added Citrate Content in Cheese and Processed Cheese Products

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Preface

This standard will replace GB/T 22031-2008<<Determination of added citrate content in cheese and processed cheese products: Enzyme - colorimetric method>>.

Appendix A and B are normative standards.

Previous edition which is replaced by this standard:

—— GB/T 22031-2008

Determination of Added Citrate Content in Cheese and Processed Cheese Products

1 Scope

This method specifies determination method for the added citrate content (expressed as citric acid) in cheese and processed cheese products.

This method is applicable for the determination of added citrate content in cheese and processed cheese products.

2 Cited Normative Documents

The reference documents cited in this standard are indispensable to this standard. For the dated references, only the dated one are applicable and for undated references, the latest edition (including revised versions) is applicable for this standard.

3 Principle

When determining the total citrate content in the sample, the added citrate content is obtained by deducting the citrate content (converted by 0.04 times of the lactose amount) carried into the sample by raw material, while citrate is expressed as citric acid.

4 Analysis Procedure

The determination of total citrate and calculation of its content refer to Appendix A.

The determination of lactose and calculation of its content refer to Appendix B.

5 Result Calculation

The added citrate content in sample is expressed and calculated as formula (1):

$$w_a = w_c - rw_i \quad \cdots\cdots(1)$$

Where:

ω_a -- Added citrate expressed in citric acid in mass(%;)

ω_c- Total citric acid in the sample, expressed in mass (%)

ω_i -- Lactose content in the sample, expressed in mass (%);

r—Ratio between citric acid content and lactose content in the raw material--whey powder or milk powder (r=0.04);

Two significant digits are reserved for the calculation results obtained from two independent test under the same condition.

6. Precision

The difference between the absolute values of the two independent determination results obtained under the repeatability condition must not exceed 10% of the average arithmetic value.

Appendix A

(Normative Appendix)

Determination of Added Citrate Content in Cheese and Processed Cheese Products- Enzyme Method

A.1 Scope

This appendix is applicable for the determination of added citrate content in cheese and processed cheese products with enzyme method.

A.2 Principle

CL converts citrate into oxaloacetate and acetate, while L-malic acid and L-lactic acid are respectively converted through catalyzed decarboxylation of oxaloacetate, which is conducted through MDH and LDH in the presence of NADH and pyruvate (decarboxylation product). During the reaction, NADH is oxidized into NAD^+. Determine the absorbance difference of NADH and calculate the citrate content in sample at 340nm.

A.3 Reagent

Unless otherwise noted, all the reagents are analytically pure. The test water should comply with the provisions in GB/T 6682.

A.3.1 Trichloroacetic acid solution (200 g/L): Weigh 20g trichloroacetic acid (CH_3COOH), dissolve in the water, blend and dilute to volume of 100mL.

A.3.2 NaOH solution (200g/L): Weigh 20g NaOH, dissolve in the water, transfer it into a 100mL volumetric flask, dilute to volume and blend.

A.3.3 NaOH solution (40g/L): Weigh 4g NaOH, dissolve in the water, transfer it into a 100mL volumetric flask, dilute to volume and blend.

A.3.4 NaOH solution (4g/L): Weigh 0.4g NaOH, dissolve in the water, transfer it into a 100mL volumetric flask, dilute to volume and blend.

A.3.5 $ZnCl_2$ solution (?g/L): Weigh 0.8g $ZnCl_2$, dissolve in the water, transfer into a 1000mL volumetric flask, dilute to volume and blend.

A.3.6 Buffer solution (pH 7.8): Weigh 7.18g $H_2NCH_2CONHCH_2CO_2H$, dissolve in 70mL water, transfer it into a 100mL volumetric flask, adjust pH to 7.8 with NaOH solution (A.3.2), add 10ml ZnCl solution (A 3.5), dilute to volume and blend. Buffer solution can be stored in refrigerator (0~4℃) for 4 weeks.

A.3.7 $NaHCO_3$ solution (4.0g/L): Weigh 4.0g $NaHCO_3$, dissolve in water, transfer into a 1000mL volumetric flask, dilute to volume and blend.

A.3.8 NADH solution: Weigh 50mg $C_{21}H_{27}N_7O_{14}P_2Na_2$ and 100mg $NaHCO_3$, dissolve in 10mL water.

A.3.9 $(NH_4)_2SO_4$ solution (422g/L): Weigh 42.2g $(NH_4)_2SO_4$, dissolve in water, transfer into a 100mL volumetric flask, dilute to volume and blend completely.

A.3.10 MDH and LDH suspension liquid: Respectively dissolve MDH (porcine heart, EC 1.1.1.37) and LDH (rabbit meat, EC 1.1.1.27) in $(NH_4)_2SO_4$ solution (A.3.9) to make the activity of MDH≥600IU/mL

and that of LDH≥1400IU/mL. After having been slowly stirred into suspension liquid, this solution can be stored in refrigerator (0~4℃) for one year.

A.3.11 CL solution: Dissolve CL in water at 0℃ (enterobacter aerogenes, EC 4.1.3.6) to make activity of MDH≥40 IU/mL. After having been slowly stirred into suspension liquid, this solution can be stored for one week in refrigerator (0~4℃) and four weeks in refrigerator at –20℃.

A.3.12 Standard citric acid solution (160μg/mL): Weigh 175mg $C_6H_8O_7.H_2O$, dissolve in water, transfer into a 1000mL volumetric flask, dilute to volume and blend completely

A.4 Instrument

Common used laboratory instruments and other items as follows.

A.4.1 Analytical balance: sensitive quantity=0.1mg

A.4.2 pH meter: accurate to ±0.1.

A.4.3 Grinder

A.4.4 Graduated colorimetric tube with stopper: 10mL, division value is 0.1mL.

A.4.5 Medium-speed filter paper

A.4.6 UV-Vis spectrophotometer, 340nm, 1cm colorimetric dish

A.4.7 Water bath pot.

A.5 Sample preparation

A.5.1 Cheese

Remove the outer layer or moldy surface to make the sample representative. Use grinder (A.4.3) to crush the sample and blend.

A.5.2 Processed cheese product

Select representative sample, use grinder (A.4.3) to crush the sample and blend.

A.6 Analysis procedure

A.6.1 Test solution preparation

Weigh 1g sample (A.5), accurate to 0.1mg, dissolve it in warm water (40~50℃), transfer into a 100mL volumetric flask completely and cool it to 20℃. Then add 1mL trichloroacetic acid (A.3.1), dilute to volume with water, blend and settle for 30min. Filter with filter paper (A.4.5), dispose 10mL primary filtrate, suck 25mL filtrate into a beaker, use NaOH solution (A.3.3) to adjust pH value of the filtrate to 4 and then use NaOH solution (A.3.4) to adjust pH value of the filtrate to 8. Transfer the solution from the beaker into 100mL volumetric flask, dilute to volume with water and simultaneously conduct blank test.

A.6.2 Determination

A.6.2.1 Drawing standard curve

Recover the temperature of the reagent to room temperature. Accurately suck two groups of standard citric acid solution(A.3.12) of 0, 0.50, 1.00, 1.50, 2.00mL, respectively put them into 10mL

colorimetric tube (A.4.4), respectively add 1.00mL buffer solution (A.3.6), 0.10mL NADH solution (A.3.8), 0.02mL MDH and LDH suspension liquid (A3.10), shake well, settle for 5 min in water bath pot (A.4.7) at 20~25℃, dilute to volume of 3.00mL. For one group, use air as the reference and adopt 1cm colorimetric dish (A.4.6) to determine the absorbance A_0 of the solution in each colorimetric tube at the wavelength of 340nm. For another group, respectively add 0.02mL CL (A.3.11), blend, settle for 5 min in water bath pot (A.4.7) at 20~25℃, use air as the reference and adopt 1cm colorimetric dish (A.4.6) to determine absorbance A_0 of the solution in each colorimetric tube at the wavelength of 340nm. Calculate the absorbance A in accordance with formula (A.1) and draw standard curve by using the citric acid content as ordinate and the absorbance as abscissa.

$$A = A_0 - A_{10} \qquad \text{(A.1)}$$

Where:

A_0—Absorbance before adding CL.
A_{10}—Absorbance after adding CL and putting into water bath for 10min.

A.6.2.2 Determination of test solution absorbance

Use pipette to suck two proportions of 2.00mL test solution (A.6.1) and put into 10mL colorimetric tube. The following procedures are as same as “Respectively add 1.00mL buffer solution (A.3.6)…. Calculate the absorbance A in accordance with formula (1)” in A.6.2.1, find the corresponding citric acid content on standard curve and simultaneously conduct blank test.

A.7 Result calculation

Citric acid content in the sample is expressed by g/100g and calculated in accordance with formula (2):

$$X = \frac{c \times V_1 \times V_3}{10\,000\, m \times V_2 \times V_4} \qquad \text{(A.2)}$$

Where:

c—Citric acid content in test solution found on standard curve. The unit is μg;
V_1—Constant volume of the sample after deproteinization, 100mL;
V_3—Constant volume of filtrate, 100mL;
m—Sample mass. The unit is g;
V_2—Volume of the sucked filtrate, 25.00mL;
V_4—Volume of the sucked test solution, 2.00mL;
Deduct blank from the calculation result, three significant digits should be reserved.

A.8 Precision

The difference between the absolute values of the two independent determination results obtained under the repeatability condition must not exceed 10% of the average arithmetic value.

Appendix B

(Normative Appendix)

Determination of Added Lactose Content in Cheese and Processed Cheese Products - Enzyme Method

B.1 Scope

This appendix is applicable for the determination of lactose content in cheese and processed cheese products with enzyme method.

B.2 principle

Under catalysis of β-GLS, lactose decomposes into D-glucose (G) and GL. HK phosphorylates D-glucose into G6P and simultaneously converts ATP into ADP. Under catalysis of G6PDH, G6P is oxidized into GA6P and NADP+ is simultaneously reduced into NADPH. Absorbance value of NADPH is proportional to the lactose content. The constant quantity should be compared with standard series.

$$C_{12}H_{22}O_{11}(L) + H_2O \xrightarrow{\beta\text{-GLS}} D\text{-}C_6H_{12}O_6(G) + D\text{-}C_6H_{12}O_6(GL)$$

$$D\text{-}C_6H_{12}O_6(G) + ATP \xrightarrow{HK} G6P + ADP$$

$$G6P + NADP^+ + H_2O \xrightarrow{G6PDH} GA6P + NADPH + H^+$$

B.3 Reagent and material

Unless otherwise noted, all the reagents are analytically pure. The test water should comply with the provisions in GB/T 6682.

B.3.1 Standard lactose ($C_{12}H_{22}O_{11}.H_2O$): Purity≥99%.

B.3.2 $K_4[Fe(CN)_5$ solution (36g/L): Weigh 3.6g $K_4[Fe(CN)_5.3H_2O]$, dissolve in water, dilute to volume of 100mL and blend.

B.3.3 $ZnSO_4$ solution (7.2g/L): Weigh 7.2g $ZnSO_4.7H_2O$, dissolve in water, dilute to volume of 100mL and blend.

B.3.4 H_2SO_4 solution (2mol/L): Dilute the concentrated H_2SO_4 with water. The ratio between the concentrated H_2SO_4 and water is 1:8 (volume ratio).

Warning: When diluting the concentrated H_2SO_4, it should be added into water slowly and agitate continuously. Otherwise, explosion will be induced.

B.3.5 NaOH solution (200g/L): Weigh 20.0g NaOH, dissolve in water, dilute to volume of 100mL and blend.

B.3.6 NaOH solution (4g/L): Weigh 4.0g NaOH, dissolve in water, dilute to volume of 1000mL and blend.

B.3.7 $(NH_4)_2SO_4$ solution (422 g/L): Weigh 42.2g $(NH_4)_2SO_4$, dissolve in water, dilute to volume of 100mL and blend.

B.3.8 Citrate buffer solution (pH 6.6): Weigh 2.8g $C_6H_5O_7Na_3.2H_2O$, 0.042g $C_6H_5O_7.H_2O$ and 0.635g $MgSO_4.7H_2O$, dissolve in 40mL water, adjust pH to 6.6±0.1 with H_2SO_4 solution (B.3.4) or NaOH solution (B.3.6), dilute to volume of 50mL with water and blend. The solution can be stored in refrigerator (0~4℃) for 3 months. It should be settled to reach room temperature before application.

B.3.9 TEA buffer solution (pH 7.6): Respectively weigh 14.0 g $C_6H_{15}NO_3.HCl$ and 0.25g $MgSO_4.7H_2O$, dissolve in 80mL water, adjust pH to 7.6±0.1 with NaOH solution (B.3.5), dilute to volume of 100mL . After blended, the solution can be stored in refrigerator (0~4℃) for 2 months.

B.3.10 $NADP^+$-ATP-TEA buffer suspension solution: Respectively weigh 65 mg $C_{21}H_{26}N_7O_{17}P_3Na_2$ and 170mg $C_{10}H_{14}N_5O_{13}P_3Na_2$, dissolve in 30mL TEA buffer solution (B.3.9). After blended, the solution can be stored in the refrigerator (0~4℃) for 3 months. It should be settled to reach room temperature before application.

B.3.11 β-GLS-$(NH_4)_2SO_4$ suspension liquid (pH value is about 7.6): Dissolve β-GLS (escherichia coli, EC 3.2.1.23) in $(NH_4)_2SO_4$ solution (A.3.9) to make the activity of β-GLS ≥600IU/mL. After having been slowly stirred into suspension liquid, this solution can be stored in refrigerator (0~4℃) for 12 months. During the application, the vessel of the suspension liquid should be immersed in ice water.

B.3.12 HK-G6PDH-$(NH_4)_2SO_4$ suspension liquid: Dissolve hexokinase (HK, yeast, EC 2.7.1.1) and G6PDH (G6PDH, yeast, EC 1.1.1.49) in $(NH_4)_2SO_4$ solution (A.3.9) to make the activity of HK≥280 IU/mL and that of G6PDH≥140 IU/mL (25℃). After having been slowly stirred into suspension liquid, this solution can be stored in refrigerator (0~4℃) for 12 months. During the application, the vessel of the suspension liquid should be immersed in ice water.

B.3.13 Standard lactose solution [$c(C_{12}H_{22}O_{11}.H_2O)$=80 μg/mL]: Accurately weigh 0.842g standard lactose (B.3.1), which has been baked to constant weight after baking for 2h at 87℃, dissolve it in water, dilute to volume of 100mL with water and shake well. Accurately suck the above solution of 1.00mL, dilute to volume of 100mL with water and obtain the standard lactose working solution of 80 μg/mL, which is stored in refrigerator (0~4℃) and prepared for immediate use.

B.4 Instrument

Common used laboratory instruments and other items are as follows.

B.4.1 Analytical balance: sensitive quantity=0.1mg

B.4.2 Quantitative filter paper: Medium speed. Diameter=15cm.

B.4.3 Graduated colorimetric tube: 10mL, with lid.

B.4.4 Spectrophotometer, 340nm, 1cm colorimetric dish

B.4.5 Water bath pot: Maintain the temperature at 20~36℃.

B.5 Sample preparation

B.5.1. Sample preparation

Take at least 200g representative sample, blend completely and put it into a closed glass vessel.

B.5.2 Test solution preparation

Accurately weigh 1g sample into a beaker, dissolve it in warm water (40~50℃), stir it with glass rod, completely transfer the sample from the beaker into a 100mL volumetric flask, dilute to volume

with water and blend. Then add 5mL $K_4[F_3(CN)_5$ solution (B.3.2), 5mL $ZnSO_4$ solution (B.3.3) and 10mL NaOH solution (B.3.6), fully mix the solution after each time of addition, dilute to volume of 100mL with water, blend and settle for 30min. Dispose part of the primary filtrate, suck 5.00 mL filtrate into a 100mL volumetric flask, dilute to volume of 100mL with water and obtain the test solution.

B.5.3 Drawing standard curve

Suck 0, 0.20, 0.40, 0.60, 0.80 and 1.00mL standard lactose working solution (B.3.13), respectively put into colorimetric tube (B.4.3), respectively add 0.20 mL citrate buffer solution (B.3.8), 0.05mL β-GLS-$(NH_4)_2SO_4$ suspension liquid (B.3.11), shake well and maintain the temperature in water bath pot for 15min. Take out the colorimetric tube, add 1.00mL $NADP^+$-ATP-TEA buffer solution (B.3.10), 0.05mL HK-G6PDH-$(NH_4)_2SO_4$ suspension liquid (B.3.12) , shake well and maintain the temperature in water bath pot (B.4.5) for 60min, take out, cool to room temperature, dilute to volume of 5.00mL with water, shake well and settle for 5min. In 1cm colorimetric dish (B.4.4), use reagent solution not containing standard lactose solution as the reference to determine the absorbance of the solution in each colorimetric tube at the wavelength of 340nm. Draw standard curve by using the lactose content as ordinate and the absorbance as abscissa.

B.5.4 Determination of test solution absorbance

Accurately suck 1.00mL test solution (B.6.1) into colorimetric tube (B.4.3), add 0.20 mL citrate buffer solution (B.3.8), 1.00mL NADP+-ATP-TEA buffer suspension solution (B.3.10), 0.05mL HK-G6PDH-$(NH_4)_2SO_4$ suspension liquid (B.3.12) , shake well and maintain the temperature in water bath pot (B.4.5) for 60min. Then take it out, cool to room temperature, dilute to volume of 5.00mL with water, shake well, settle for 5min and use the solution as the reference of test solution.

Accurately suck 1.00mL test solution (B.6.1) into colorimetric tube (B.4.3). The following procedures are operated as “respectively add 0.20 mL citrate buffer solution (B.3.8)…. and settle for 5min” in B.6.2, determine the absorbance of the solution in each colorimetric tube at the wavelength of 340nm and find the corresponding lactose content on standard curve.

B.6 Result calculation

Lactose content in the sample is expressed by g/100g and calculated in accordance with formula (B.1):

$$X = \frac{c \times V_1 \times V_3}{10\,000\, m \times V_2 \times V_4} \qquad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (B.1)$$

Where:

c—Lactose content in test solution found on standard curve. The unit is μg;

V_1—Constant volume of the sample after deproteinization, 250mL;

V_3—Constant volume of filtrate, 100mL;

m—Sample mass. The unit is g;

V_2—Volume of the sucked filtrate, 5.00mL;

V_4—Volume of the sucked test solution, 1.00mL;

The above values should be expressed in the arithmetic mean of parallel determination. Three significant digits should be reserved.

B.7 Precision

The difference between the absolute values of the two independent determination results obtained under the repeatability condition must not exceed 10% of the average arithmetic value.