GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.83-2016

National Food Safety Standard -

Determination of Carotene in Foods

Issued on: December 23, 2016 **Implemented on: June 23, 2017**

Issued by: National Health and Family Planning Commission of the PRC;

China Food and Drug Administration.

Table of Contents

Foreword

This Standard replaces GB 5413.35-2010 “National Food Safety Standard - Determination of β-carotene in foods for infants and young children, milk and milk products”, GB/T 5009.83-2003 “Determination of carotene in foods”, and NY/T 82.15-1988 “Method for determination of fruit juice - Determination of β-carotene”.

As compared with GB 5413.35-2010, the main changes of this Standard are as follows:

- The standard’s name is changed to “National Food Safety Standard - Determination of Carotene in Foods”;

- ADD pretreatment methods for common foods;

- ADD the chromatographic conditions where α-carotene and β-carotene need to be differentiated;

- MODIFY the results expression of carotene.

National Food Safety Standard - Determination of Carotene in Foods

1 Scope

This Standard specifies the method for determination of carotene in foods.

This Standard's chromatographic condition I is applicable to the determination of α-carotene, β-carotene, and total carotene in foods. The chromatographic condition II is applicable to the determination of β-carotene in foods.

2 Principles

The sample is saponified to release the carotene to a free state. After using petroleum ether to extract dichloromethane and dilute, ADOPT reversed phase chromatography to separate and external standard method to quantify.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are analytically pure. The water is the Grade I water specified in GB/T 6682.

3.1 Reagents

3.1.1 α-amylase: Enzyme activity≥1.5 U/mg.

3.1.2 Papain: Enzyme activity≥5 U/mg.

3.1.3 Potassium hydroxide (KOH).

3.1.4 Anhydrous sodium sulfate (Na_2SO_4).

3.1.5 Ascorbic acid ($C_6H_8O_6$).

3.1.6 Petroleum ether: The boiling range is 30 °C~60 °C.

3.1.7 Methanol (CH_4O): chromatographically pure.

3.1.8 Acetonitrile (C_2H_3N): chromatographically pure.

3.1.9 Chloroform ($CHCl_3$): chromatographically pure.

3.1.10 Methyl tert-butyl ether [$CH_3OC(CH_3)_3$]: chromatographically pure.

3.1.11 Dichloromethane (CH_2Cl_2): chromatographically pure.

3.1.12 Anhydrous ethanol (C_2H_6O): guaranteed reagent.

3.1.13 N-hexane (C_6H_{14}): chromatographically pure.

3.1.14 2,6-di-tert-butyl-4-methylphenol ($C_{15}H_{24}O$, BHT).

3.2 Preparation of reagent

Potassium hydroxide solution: WEIGH 500 g of solid potassium hydroxide; ADD 500 mL of water to dissolve. Prepared before use.

3.3 Standards

3.3.1 α-carotene ($C_{40}H_{56}$, CAS number: 7488-99-5): Purity≥95%. Or a reference material which has been certified by the state and awarded a reference material certificate.

3.3.2 β-carotene ($C_{40}H_{56}$, CAS number: 7235-40-7): Purity≥95%. Or a reference material which has been certified by the state and awarded a reference material certificate.

3.4 Preparation of standard solutions

3.4.1 Standard stock solution of α-carotene (500 μg/mL): Accurately WEIGH 50 mg (accurate to 0.1 mg) of α-carotene standard; ADD 0.25 g of BHT; USE dichloromethane to dissolve; TRANSFER to a 100 mL brown volumetric flask and DILUTE to volume. PROTECT it from light at below -20 °C. The service life shall not exceed 3 months. The standard stock solution, before use, needs to be calibrated. For specific operations, SEE Appendix A.

3.4.2 Standard intermediate solution of α-carotene (100 μg/mL): Accurately PIPETTE 10.0 mL of standard stock solution of α-carotene into a 50 mL brown volumetric flask; and USE dichloromethane to dilute to volume.

3.4.3 Standard stock solution of β-carotene (500 μg/mL): Accurately WEIGH 50 mg (accurate to 0.1 mg) of β-carotene standard; ADD 0.25 g of BHT; USE dichloromethane to dissolve; TRANSFER to a 100 mL brown volumetric flask and DILUTE to volume. PROTECT it from light at below -20 °C. The service life shall not exceed 3 months. The standard stock solution, before use, needs to be calibrated. For specific operations, SEE Appendix A.

Note: The β-carotene standard is mainly all-E-β-carotene. During storage, affected by temperature, oxidation, and other factors, some all-E-β-carotene isomerizes to cis-

Liquid sample: WEIGH accurately 5 g~10 g (accurate to 0.001 g); PLACE in a 250 mL conical flask; and ADD 1 g of ascorbic acid.

5.2.2.2 Saponification

TAKE the pretreated sample; ADD 75 mL of anhydrous ethanol, SHAKE well; ADD 25 mL of potassium hydroxide solution; and CAP the bottle stopper. PLACE in a constant temperature oscillating water bath which has been preheated to 53 °C±2 °C to saponify for 30 min. REMOVE, LET stand, and COOL to room temperature.

Note: If saponification is not complete, the saponification time may be appropriately extended to 1 h.

5.3 Sample extraction

TRANSFER the saponification solution to a 500 mL separatory funnel; ADD 100 mL of petroleum ether, gently SHAKE, EXHAUST, and CAP the stopper. After oscillating at room temperature for 10 min, LET it stand for stratification; TRANSFER the aqueous phase to another separatory funnel, to perform second extraction according to the above method. COMBINE the organic phases; and USE water to wash to near-neutral. DISCARD the aqueous phase; and the organic phase is dehydrated by filtration over anhydrous sodium sulfate. The filtrate is collected in a 500 mL evaporating flask and concentrated under reduced pressure at 40 °C±2 °C on a rotary evaporator until nearly dry. USE nitrogen to blow-dry; USE a pipette to accurately add 5.0 mL of dichloromethane; CAP the stopper to fully dissolve the extract. After filtering it through a 0.45 μm membrane and discarding approximately 1 mL of the initial filtrate, COLLECT in a sample injection bottle for use.

Note: If necessary, to concentrate or dilute according to the carotene content in the sample solution to be determined, so that the concentration of α-carotene and/or β-carotene in the sample solution to be determined is in the range of 0.5 μg/mL~10 μg/mL.

5.4 Chromatographic determination

5.4.1 Chromatographic condition I (applicable to the determination of α-carotene, β-carotene, and total carotene in foods)

5.4.1.1 Reference chromatographic conditions

Reference chromatographic conditions are listed below:

a) Chromatographic column: C_{30} column; the column length is 150 mm; the inner diameter is 4.6 mm; the particle size is 5 μm; or equivalent column;

ρ - Calibration concentration of β-carotene standard working solution, in micrograms per milliliter (μg/mL);

CP - Chromatographic purity of all-E-β-carotene, %.

5.4.1.3 Sample determination

Under the same chromatographic conditions, INJECT the solution to be determined into liquid chromatograph; qualitative based on the retention time; based on the peak area, USE the external standard method to quantify. The concentration of α-carotene in the solution to be determined shall be calculated according to the standard curve regression equation. β-carotene shall be calculated based on the all-E-β-carotene response factor.

5.4.2 Chromatographic condition II (applicable to the determination of β-carotene in foods)

5.4.2.1 Reference chromatographic conditions

Reference chromatographic conditions are listed below:

a) Chromatographic column: C_{18} column; the column length is 250 mm; the inner diameter is 4.6 mm; the particle size is 5 μm; or equivalent column;

b) Mobile phase: chloroform: acetonitrile: methanol=3:12:85, containing 0.4 g/L of ascorbic acid, filtered through a 0.45 μm membrane for use;

c) Flow velocity: 2.0 mL/min;

d) Detection wavelength: 450 nm;

e) Column temperature: 35 °C±1 °C;

f) Injection volume: 20 μL.

5.4.2.2 Making of standard curve

INJECT the standard working solution of β-carotene into HPLC (for chromatogram, SEE Figure C.2); qualitative based on the retention time; DETERMINE the peak area. TAKE the concentration of standard series working solution as the abscissa, the peak area as the ordinate, to plot standard curve; and CALCULATE regression equation.

5.4.2.3 Sample determination

Under the same chromatographic conditions, INJECT the solutions to be determined into liquid chromatograph separately, to perform HPLC analysis;

in peak area (AU);

A_{9Z} - The peak area of 9-cis-β-carotene in the sample solution to be determined, in peak area (AU);

A_{13Z} - The peak area of 13-cis-β-carotene in the sample solution to be determined, in peak area (AU);

1.2 - Relative correction factor of 13-cis-β-carotene;

A_{15Z} - The peak area of 15-cis-β-carotene in the sample solution to be determined, in peak area (AU);

1.4 - Relative correction factor of 15-cis-β-carotene;

A_{xZ} - The peak area of other cis-β-carotene in the sample solution to be determined, in peak area (AU);

V - Constant volume of sample solution, in milliliters (mL);

100 - The coefficient which expresses the result in micrograms per hundred grams (μg/100 g);

RF - All-E-β-carotene response factor, in peak area milliliters per microgram (AU · mL/μg);

m - Sample mass, in grams (g).

Note 1: Due to the different percentile absorption coefficients of the isomers of β-carotene (SEE Appendix D), in the calculation for β-carotene, a relative correction factor needs to be used to correct the results.

Note 2: If the content of other cis-β-carotene in the sample is low, the calculation may not be performed.

The total carotene content in the sample shall be calculated according to the equation (4):

$$X_{total} = X_{\alpha} + X_{\beta} \qquad \cdots\cdots(4)$$

Where:

X_{total} - Total carotene content in the sample, in micrograms per hundred grams (μg/100 g);

X_{α} - The content of α-carotene in the sample, in micrograms per hundred grams (μg/100 g);

Appendix B

Confirmation of retention time of β-carotene isomers and calculation of chromatographic purity of all-E-β-carotene

Note: When chromatographic condition I is used for the determination of β-carotene, the retention time of β-carotene isomers needs to be determined, and the chromatographic purity of β-carotene standard solution shall be corrected.

B.1 Reagent

Iodine solution (I_2): 0.5 mol/L.

B.2 Preparation of reagents

B.2.1 Iodohydrin solution (0.05 mol/L): PIPETTE 5 mL of iodine solution; USE ethanol to dilute to 50 mL, and MIX well.

B.2.2 Isomerized β-carotene solution: TAKE 10 mL of standard stock solution of β-carotene in a beaker; ADD 20 μL of iodohydrin solution; SHAKE well and IRRADIATE it in sunlight or 30 cm away from 40 W fluorescent lamp for 15 min; USE dichloromethane to dilute to 50 mL. SHAKE well and FILTER by a 0.45 μm membrane for HPLC chromatographic analysis.

B.3 Confirmation of retention time of β-carotene isomers

Separately TAKE standard intermediate solution of β-carotene (100 μg/mL) and isomerized β-carotene solution; according to chromatographic condition I, INJECT them into HPLC for chromatographic analysis. According to the chromatogram of standard intermediate solution of β-carotene, CONFIRM the retention time of all-E-β-carotene. COMPARE the peak area changes in the chromatograms of standard intermediate solution of β-carotene and isomerized β-carotene solution, and the positional relationship with all-E-β-carotene, to confirm the retention time of cis-β-carotene isomers: A larger chromatographic peak before all-E-β-carotene is 13-cis-β-carotene; a larger chromatographic peak immediately after all-E-β-carotene is 9-cis-β-carotene; 15-cis-β-carotene is followed by 13-cis-β-carotene; and there may be other smaller cis-structure chromatographic peaks. The chromatogram is shown in Figure C.1.

B.4 Calculation of chromatographic purity of all-E-β-carotene standard solution

TAKE standard working solution of β-carotene (3 μg/mL); according to

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------