NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.83-2016

Replacing GB 5413.35-2010; GB/T 5009.83-2003; NY/T 82.15-1988

National food safety standard - Determination of carotene in foods

食品安全国家标准 食品中胡萝卜素的测定

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Standardization Administration of the PRC

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Amendment No. 1

2 Principle

2 The sample is saponified so that the carotene is released in the free form, it is extracted with petroleum ether and made up to volume in dichloromethane, then separated by reversed-phase chromatography and quantified against an external standard.

2 (Amendment No. 1) Item one of Amendment No. 1 replaces the wording the sample is saponified so that the carotene is released in the free form with the wording the sample is treated.

3 Reagents and materials

3 Unless otherwise stated the reagents used in this method are of analytical grade and the water is grade one water as specified in GB/T 6682.

3.1 The reagents listed are alpha-amylase with an enzyme activity of at least 1.5 U/mg; papain with an enzyme activity of at least 5 U/mg; potassium hydroxide; anhydrous sodium sulfate; ascorbic acid; petroleum ether with a boiling range of 30 °C to 60 °C; methanol, acetonitrile, trichloromethane, methyl tert-butyl ether, dichloromethane and n-hexane, all of chromatographic grade; absolute ethanol of guaranteed grade; and butylated hydroxytoluene.

3.2 The potassium hydroxide solution is prepared by dissolving 500 g of solid potassium hydroxide in 500 mL of water, and is prepared just before use.

3.3 The standards are alpha-carotene, CAS number 7488-99-5, and beta-carotene, CAS number 7235-40-7, each of purity not less than 95 %, or a certified reference material with a national certificate.

3.4.1 The alpha-carotene standard stock solution of 500 μg/mL is prepared by weighing 50 mg of the alpha-carotene standard to 0.1 mg, adding 0.25 g of butylated hydroxytoluene, dissolving in dichloromethane and making up to the mark in a 100 mL brown volumetric flask. It is stored below minus 20 °C in the dark and used within 3 months. The stock solution is standardized before use as described in Annex A.

3.4.3 The beta-carotene standard stock solution of 500 μg/mL is prepared in the same way from 50 mg of the beta-carotene standard, stored in the same way and standardized in the same way. A note states that the beta-carotene standard is mainly all-trans beta-carotene and that during storage, under the influence of temperature, oxidation and other factors, part of it isomerizes to cis forms such as 9-cis, 13-cis and 15-cis beta-carotene; where beta-carotene is determined under chromatographic conditions one, the retention times of the beta-carotene isomers shall be confirmed according to Annex B and the

chromatographic purity of the all-trans beta-carotene standard solution shall be calculated.

3.4.5 The mixed alpha-carotene and beta-carotene working standard solutions for chromatographic conditions one are prepared by taking 0.50 mL, 1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL and 10.00 mL of the alpha-carotene standard intermediate solution into six 100 mL brown volumetric flasks, adding 3.00 mL of the beta-carotene intermediate solution to each and making up to the mark with dichloromethane, which gives alpha-carotene concentrations of 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL, 3.0 μg/mL, 4.0 μg/mL and 10.00 μg/mL with a beta-carotene concentration of 3.0 μg/mL throughout.

5 Analytical procedure

5 A note states that light shall be excluded throughout the experimental work.

5.1 Cereals, pulses, nuts and similar samples are crushed, ground and sieved through a sieve plate with an aperture of 0.3 mm to 0.5 mm; vegetables, fruit, eggs, algae and similar samples are homogenized; solid powders and liquid samples are shaken or stirred until uniform before use. Samples may be kept for one week in a refrigerator at 4 °C.

5.1 (Amendment No. 1) Item two of Amendment No. 1 adds, after the sentence on vegetables, fruit, eggs and algae, that hard candy and similar samples are crushed in a mill or a homogenizer and then mixed, and that gel candy and other soft sweets are cut up with scissors and then mixed.

5.2.1.1 For ordinary food samples such as vegetables, fruit, mushrooms and algae, cereals, pulses and eggs, 1 g to 5 g of the well mixed sample is weighed to 0.001 g, or 0.2 g to 2 g for oils, into a 250 mL conical flask; 1 g of ascorbic acid and 75 mL of absolute ethanol are added and the flask is shaken in a water bath at 60 °C +/- 1 °C for 30 min. Where the sample is high in protein or starch, above 10 %, 1 g of ascorbic acid, 15 mL of warm water at 45 °C to 50 °C, 0.5 g of papain and 0.5 g of alpha-amylase are added first, the flask is stoppered and mixed and held in a thermostatic water bath at 55 °C +/- 1 °C with shaking or ultrasound for 30 min, and only then are the 75 mL of absolute ethanol added and the flask shaken in the water bath at 60 °C +/- 1 °C for 30 min.

5.2.1.2 For saponification, 25 mL of potassium hydroxide solution is added, the flask is stoppered and placed in a thermostatic shaking water bath preheated to 53 °C +/- 2 °C, and saponification is carried out for 30 min. The flask is then taken out, left to stand and cooled to room temperature.

5.2.2.1 For samples to which beta-carotene has been added, a solid sample of 1 g to 5 g is weighed to 0.001 g into a 250 mL conical flask, 1 g of ascorbic acid and 50 mL of warm water at 45 °C to 50 °C are added and mixed, then 0.5 g of papain and 0.5 g of alpha-amylase are added, alpha-amylase being omitted for a starch free sample, and the stoppered flask is held in a thermostatic water bath at 55 °C +/- 1 °C with shaking or ultrasound for 30 min. A liquid sample of 5 g to 10 g is weighed to 0.001 g into a 250 mL

conical flask and 1 g of ascorbic acid is added.

5.2.2.1 (Amendment No. 1) Item three of Amendment No. 1 changes solid sample to solid sample, other than solid beverages and candy, and adds that for solid beverages and candy 0.2 g to 5 g of the sample is weighed to 0.001 g into a 250 mL conical flask, 1 g of ascorbic acid and 50 mL of water are added and mixed, the flask is shaken or treated with ultrasound for 30 min, and extraction then follows 5.3.

5.2.2.2 The pre-treated sample is saponified by adding 75 mL of absolute ethanol, shaking, then adding 25 mL of potassium hydroxide solution, stoppering the flask and placing it in a thermostatic shaking water bath preheated to 53 °C +/- 2 °C for 30 min; the flask is then taken out, left to stand and cooled to room temperature. A note states that where saponification is incomplete the time may be extended to 1 h.

5.3 The saponified liquid is transferred to a 500 mL separating funnel, 100 mL of petroleum ether is added, the funnel is swirled gently, vented, stoppered and shaken for 10 min at room temperature and then left to separate; the aqueous phase is transferred to another separating funnel and extracted a second time in the same way. The organic phases are combined and washed with water until nearly neutral. The aqueous phase is discarded and the organic phase is dried by filtration through anhydrous sodium sulfate. The filtrate is collected in a 500 mL evaporating flask and concentrated to near dryness under reduced pressure on a rotary evaporator at 40 °C +/- 2 °C, then blown dry with nitrogen. Exactly 5.0 mL of dichloromethane is added with a pipette, the flask is stoppered and the extract fully dissolved, the solution is filtered through a 0.45 μm membrane, the first millilitre or so of the filtrate is discarded and the rest is collected in a sample vial. A note states that the test solution may be concentrated or diluted as the carotene level requires, so that the alpha-carotene or beta-carotene concentration lies between 0.5 μg/mL and 10 μg/mL.

5.3 (Amendment No. 1) Item four of Amendment No. 1 replaces the words the saponified liquid with the words the treated sample liquid.

5.4.1.1 Chromatographic conditions one, which serve for alpha-carotene, beta-carotene and total carotene, are as follows: a C30 column 150 mm long, 4.6 mm in internal diameter and of 5 μm particle size, or an equivalent column; mobile phase A of methanol, acetonitrile and water in the ratio 73.5 to 24.5 to 2 and mobile phase B of methyl tert-butyl ether, with the gradient of Table 1; a flow rate of 1.0 mL/min; a detection wavelength of 450 nm; a column temperature of 30 °C +/- 1 °C; and an injection volume of 20 μL. Table 1 gives the gradient programme in three rows: at 0 min, 15 min, 18 min, 19 min, 20 min and 22 min the proportion of mobile phase A is 100 %, 59 %, 20 %, 20 %, 0 % and 100 % and the proportion of mobile phase B is 0 %, 41 %, 80 %, 80 %, 100 % and 0 %.

5.4.1.2 The alpha-carotene calibration curve is drawn from the concentrations of the series of working standard solutions and the corresponding peak areas, with concentration on the horizontal axis and peak area on the vertical axis, and the regression equation is