GB 5009.210-2016 National food safety standard  Determination of Pantothenic Acid in Foods

GB 5009.210-2016 食品安全国家标准 食品中泛酸的测定

NHFPC
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National Standard of the People’s Republic of China

GB 5009.210–2016

National Food Safety Standard

Determination of Pantothenic Acid in Foods

食品安全国家标准

食品中泛酸的测定

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Preface

This standard replaces GB/T 5009.210–2008 “Determination of Pantothenic Acid in Foods” and GB 5413.17–2010 “National Food Safety Standard Determination of Pantothenic Acid in Foods for Infants and Young Children, Milk and Milk Products.” Compared with GB/T 5009.210–2008, major changes of this standard are as follows:

——the title of the standard has been revised to “National Food Safety Standard Determination of Pantothenic Acid in Foods”;
——the HPLC method has been added;
——the pretreatment method for foods has been revised;
——the limit of detection and the quantitation limit have been added;
National Food Safety Standard
Determination of Pantothenic Acid in Foods

1 Scope

This standard specifies the method for determination of pantothenic acid and calcium pantothenate in foods.

Method I in this standard applies to the determination of pantothenic acid in foods; Method II applies to the determination of pantothenic acid and calcium pantothenate in the nutrition supplements of health food and formula food.

Method I Microbiological Method

2 Principles

Pantothenic acid is a nutrient essential for the growth of *Lactobacillus plantarum* (ATCC 8014) and under certain controlled conditions, inoculate the *Lactobacillus plantarum* solution into the culture solution containing test sample solution and incubate for a certain time to determine the light transmittance (or absorbance value) and then calculate the content of pantothenic acid in the test sample according to the standard curve of pantothenic acid content and light transmittance (or absorbance value).

3 Reagents and Materials

Unless otherwise specified, all the reagents used in this method are analytical reagents, and the water is the Grade 2 water stipulated in GB/T 6682.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl);
3.1.2 Glacial acetic acid (C₂H₄O₂);
3.1.3 Sodium hydroxide (NaOH);
3.1.4 Sodium chloride (NaCl);
3.1.5 Sodium carbonate (Na₂CO₃);
3.1.6 Potassium bicarbonate (KHCO₃);
3.1.7 Dipotassium hydrogen phosphate (K₂HPO₄);
3.1.8 Sodium acetate trihydrate (C₂H₃O₂Na·3H₂O);
3.1.9 Potassium dihydrogen phosphate trihydrate (KH₂PO₄·3H₂O);
3.1.10 Magnesium sulfate heptahydrate (MgSO₄·7H₂O);
3.1.11 Ferrous sulfate heptahydrate (FeSO₄·7H₂O);
3.1.12 Manganese sulfate (MnSO₄·H₂O);
3.1.13 [Tri (hydroxymethyl) aminomethane (C₄H₁₁NO₃);
3.1.14 Glucose (C₆H₁₂O₆);
3.1.15 Methylbenzene (C₇H₈);
3.1.16 Absolute ethanol (C₂H₆O);
3.1.17 Anion exchange resin Dowex 1×8: with the particle size of 38μm~75μm;
3.1.18 Alkaline phosphatase: with the enzyme activity ≥ 23U/g;
3.1.19 Liver acetone powder, from pigeon: with the enzyme activity ≥ 0.1U/g;
3.1.20 Peptone: with the nitrogen content ≥ 10%;
3.1.21 Yeast extract: with the nitrogen content ≥ 10%;
3.1.22 Agar;

3.2 Reagent preparation

3.2.1 Acetic acid solution (0.2mol/L): pipette 11.8mL of glacial acetic acid, and then dilute it to 1000mL with water and mix it well;
3.2.2 Sodium acetate solution (0.2mol/L): weigh 27.2g of sodium acetate trihydrate, add water to dissolve it, and then dilute it to 1000mL and mix it well;
3.2.3 Hydrochloric acid solution (1mol/L): pipette 83mL of hydrochloric acid, and then dilute it to 1000mL with water and mix well;
3.2.4 Hydrochloric acid soak solution: pipette 100mL of hydrochloric acid and mix with 50 times of water;
3.2.5 Sodium hydroxide solution (1mol/L): weigh 40g of sodium hydroxide, add water to dissolve, and then dilute it to 1000mL and mix it well;
3.2.6 Sodium hydroxide solution (0.1mol/L): weigh 4g of sodium hydroxide, add water to dissolve it, and then dilute it to 1000mL and mix well;
3.2.7 Tris buffer solution: weigh 121.0g of [Tri (hydroxymethyl) aminomethane], dissolve it in 500mL of water, adjust the pH to 8.1±0.1 with glacial acetic acid, and then add water to 1000mL, and mix well; This solution can be kept for two (2) weeks at 2℃~4℃ in a refrigerator.
3.2.8 Physiological saline: weigh 9g of sodium chloride, add water to dissolve and then dilute it to 1000mL and mix well. Before use, conduct the autoclaved sterilization for 10min at 121℃ for later use.
3.2.9 Ethanol solution (20%): measure 200mL of absolute ethanol and mix it with 800mL of water;
3.2.10 Sodium carbonate solution (0.08mol/L): weigh 8.5g of sodium carbonate, add water to dissolve and then dilute it to 1000mL and mix well;
3.2.11 Potassium bicarbonate solution (0.02mol/L): weigh 2g of potassium bicarbonate, add water to dissolve, and then dilute it to 1000mL and mix well;
3.2.12 Alkaline phosphatase solution: weigh 2g of alkaline phosphatase, add water to dissolve, and then dilute it to 100mL. Immediately prepare before use. Keep it at 2℃~4℃ refrigerator.
3.2.13 Pigeon liver extract solution
3.2.13.1 Activated Dowex 1×8: weigh 100g of Dowex 1×8 into a conical flask, add 1L of hydrochloric acid solution and fully shake it for 10min on the oscillator, and filter with Buchner funnel with filter paper. Transfer the Dowex 1×8 into the conical flask, add 1L of hydrochloric acid solution, shake repeatedly and filter. Add 1L of water to Dowex 1×8 and shake it for 10min, filter and wash for ten (10) times with water. Dropwise add Tris buffer solution and adjust the pH of Dowex 1×8 to 8.0±0.1. Keep it at 2℃~4℃ refrigerator, and use it up within two (2) days.
3.2.13.2 Pigeon liver extract solution: on the first day before preparing this reagent, keep the container used at 2℃~4℃ refrigerator for overnight. Weigh 30g of pigeon liver acetone powder into a mortar; under the condition of ice bath, add 300mL of potassium bicarbonate solution in two times, and grind it to homogenate; transfer it to a centrifugal tube with stopper, cover the plug and fully shake it to mix well. Freeze it at −20℃ for 10min, and centrifuge for 5min at the speed of 3000r/min, then transfer the supernatant liquid to a 500–mL jar. Add 150g of activated Dowex 1×8, shake it for 5min in the ice bath and pour the mixed solution into the centrifugal tube and then centrifuge for 5min at the speed of 3000r/min. Transfer the supernatant liquid to another 500–mL pre–cold jar, and freeze it for 10min at −20℃; add 150g of activated Dowex 1×8, shake it for 5min in the ice bath and pour the mixed solution into the centrifugal tube and then centrifuge at the speed of 3000r/min; Respectively place the supernatant liquid in test tubes with stopper (about 3mL for each tube). Keep it frozen at −20℃. Before use, defrost it and keep it at 2℃~4℃ refrigerator.

3.3 Culture medium

3.3.1 Salt solution A: respectively weigh 25g of dipotassium hydrogen phosphate and 25g of potassium dihydrogen phosphate trihydrate, add water to dissolve and then dilute to 500mL and mix well. Add 1mL of methylbenzene and it can be kept at 2℃~4℃ refrigerator for one (1) year.
3.3.2 Salt solution B: respectively weigh 10g of magnesium sulfate heptahydrate, 0.5g of sodium chloride, 0.5g of ferrous sulfate heptahydrate and 0.5g of manganese sulfate monohydrate, add water to dissolve and dilute to 500mL. Add 5 drops of hydrochloric acid, and it can be kept at 2℃~4℃ refrigerator for one (1) year.

3.3.3 Agar culture medium: weigh or pipette each reagent as per amount of Table 1, add water to 100mL, mix well and heat up on boiling water bath for complete dissolution of agar; when it is still hot, adjust the pH to 6.8±0.1 with 1mol/L hydrochloric acid solution and/or 1mol/L sodium hydroxide solution. Immediately put it into different test tubes with 3mL~5mL for each tube (depending on the different inner diameter of test tubes), and the liquid height shall not be less than 2cm. Cover the cotton plug, perform autoclaved sterilization for 15min at 121℃; take the tube out, and make the test tube upright, cool the tube down and then keep the tube in refrigerator for later use.

Table 1 List for Preparation of Agar Culture Medium for Stock Strain

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>1.0g</td>
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<tr>
<td>Peptone</td>
<td>0.8g</td>
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<tr>
<td>Yeast extract powder</td>
<td>0.2g</td>
</tr>
<tr>
<td>Sodium acetate trihydrate</td>
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<tr>
<td>Salt solution A</td>
<td>0.2mL</td>
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<tr>
<td>Salt solution B</td>
<td>0.2mL</td>
</tr>
<tr>
<td>Agar</td>
<td>1.2g</td>
</tr>
</tbody>
</table>

3.3.4 Culture solution for determination of pantothenic acid: be prepared according to Annex A; or culture medium with equivalent effect may be purchased from Reagent Company and be prepared as instructed before use.

3.4 Standard substances

D–calcium pantothenate (C_{18}H_{32}CaN_{2}O_{10}): with the purity ≥ 99%.

3.5 Preparation of standard solutions

3.5.1 Pantothenic acid standard stock solution (40.0μg/mL): accurately weigh 43.5mg of D–calcium pantothenate that has been pre-dried to constant weight, add water to dissolve and then transfer it into a 1000–mL volumetric flask, add 10mL of acetic acid solution and 100mL of sodium acetate solution, and dilute to
volume with water. Keep it in a brown flask and then add 3~5 drops of methylbenzene and it can be kept at 2°C~4°C refrigerator for two (2) years.

3.5.2 Pantothenic acid standard intermediate solution (1.00μg/mL): accurately pipette 25.0mL of pantothenic acid standard stock solution into a 1000-mL volumetric flask; add 10mL of acetic acid solution and 100mL of sodium acetate solution, and dilute to volume with water. Add 3~5 drops of methylbenzene and it can be kept at 2°C~4°C refrigerator for one (1) year.

3.5.3 Pantothenic acid standard working solution (20ng/mL): accurately pipette 2.00mL of pantothenic acid standard intermediate solution into a 100-mL volumetric flask, and then dilute it to volume with water and mix well. This solution shall be immediately prepared before use.

4 Apparatus and Equipment

4.1 Balance: with the sensitivity of 0.1mg.
4.2 Constant temperature incubator: 37°C±1°C;
4.3 Pressure steam disinfector: 121°C;
4.4 Vortex oscillator;
4.5 Centrifuge: with a rotating speed ≥ 3000r/min;
4.6 Inoculating needle and inoculating loop;
4.7 pH meter: with the precision of ±0.01;
4.8 Ultraviolet spectrophotometer;
4.9 Clean bench;
4.10 Ultrasonic oscillator;

5 Preparation and Storage of Strains

5.1 Strain: *Lactobacillus plantarum* (ATCC 8014).

5.2 Preparation of stock strain

Inoculate *Lactobacillus plantarum* (ATCC 8014) into agar culture medium, incubate it for 20h~24h at the constant temperature incubator (37°C±1°C), transfer and inoculate it continuously for 2~3 times. Take it out and store at 2°C~4°C refrigerator as stock strain; Subculture cells at least once a month (not exceeding 25 generations);
Before conducting the experiment, inoculate the stock strain into the agar culture medium, and incubate at constant temperature incubator (37℃±1℃) for 20h~24h so as to activate the strains for the preparation of inoculated solution.

Note: The stock strain kept for several weeks cannot be immediately used for the preparation of inoculated solution; instead, 2~3 generation inoculation shall be continuously conducted before use, so as to ensure the activity of strains.

5.3 Preparation of inoculum

On the day before test, mix 2mL of pantothenic acid standard working solution and 4mL of culture solution for determination of pantothenic acid and then separately fill in two 5–mL centrifuge tubes, cover the cotton plug and go on autoclaved sterilization for 15min at 121℃; The seed culture solution is obtained. After cooling down, transfer and inoculate the activated strains to two tubes of seed culture solution with inoculating loop, and incubate at the constant temperature incubator (37℃±1℃) for 20h~24h; Take it out and then centrifuge for 10min (3000r/min) and remove the supernatant liquid. Wash two times with the pre-sterilized physiological saline under sterile operation, centrifuge for 10min (3000r/min) and remove the supernatant liquid and then add 3mL of sterile physiological saline and oscillate to mix well to get the inoculated solution for an immediate use.

6 Analysis Procedures

Note: All the operations shall be performed away from light.

6.1 Test sample preparation

For test sample of cereals & potatoes, beans, and milk powder, etc., it is required to be smashed, grinded and sieved (with the sieve plate aperture of 0.3mm~0.5mm); for meat, eggs and nuts, etc., it is required to be processed to chyme with homogenizer; for test sample of fruit and vegetable, and semi-solid food, etc., it is required to be homogenized well; for liquid test sample, it is required to be shaken to mix well before use. The test sample can be kept for one week at 4°C refrigerator;

6.2 Test sample extraction
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<thead>
<tr>
<th>Membership</th>
<th>Information</th>
<th>Knowledge</th>
<th>Database</th>
<th>Training</th>
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<td>Alerts</td>
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