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Silkworm Pupae Peptone Culture Medium Materials

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Introduction

This standard is proposed by Beijing Hongrun Baoshun Technology Co., LTD. This standard is drafted by Beijing Hongrun Baoshun Technology Co., LTD. The drafters of this standard are PU Qiangchun (Mr.), MIU Fengguang (Mr.). This standard was first issued at November 2018.

Silkworm Pupae Peptone Culture Medium Materials

1 Scope

This standard specifies the requirements, test methods, inspection rules, labeling, packaging, transportation, and storage of the silkworm pupae peptone of culture medium materials. This standard is applicable to the silkworm pupae peptone of culture medium materials.

2 Normative references

The following documents are essential to the application of this document. For dated references, the dated version only applies to this document. For undated references, the latest version (including all amendments) applies to this document.

GB 12801-2008 General principles for safety and hygiene in the production process
GB 15981-1995 Evaluation methods and standards of disinfection and sterilization effacts
WS/T 232-2002 Procedures for the quality inspection of commercial microbial medium
WS 233-2002 Common Criteria for biosafety in microbiology and biomedical laboratories
GB 9687-1988 Hygienic standards for polyethylene molding of food packaging.
Manual of new technical standards and procedures for food hygiene inspection (2004)
Pharmacopoeia of the People's Republic of China (2015 edition) three
Measures for supervision and administration of quantitative packaged goods measurement, the 75th order by General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ)

3 Definitions

The following definitions apply to this standard.

3. 1 Silkworm Pupae Peptone

It is the basic raw materials of the fermentation industry and various microbial medium.

Silkworm pupae peptone is a dried powder refined by high quality silkworm pupaes through the biological extraction process. The production process shall comply with GB 12801-2008 and GB 15987-1995.

3. 2 Culture medium materials

It refers to the nutrient used for the isolation, identification and preservation of microorganisms.

3.3 Loss on drying

It refers to all water that has evaporated from the object under 105°C. This test shall comply with the provisions of WS/T 232-2002.

3.4 Residue

It refers to the weight of the inorganic substance left after burning the reagent to constant weight under 700-800 $^{\circ}$ C.

3.5 Total nitrogen

Kjeldahl method was used to determine the total nitrogen in protein.

3. 6 Amino nitrogen

It is to determine the content of peptide of free ammonia peptide nitrogen in hydrolyzed protein. This method shows the hydrolysis degree of protein.

- 4 Requirements
- 4.1 Sensory index

It is a light yellow powder and has a special smell of silkworm pupae peptone, easy to absorb moisture and solve in water.

4. 2 Performance index

See table 1

Description	Requirements
Loss on drying	≤8.0%
Residue	≤12.0%
Total nitrogen	≥13.5%
Amino nitrogen	≥2.0%

Table 1

4.3 Net content

The net content shall comply with the regulation of the 75th order by AQSIQ. The net content of the same batch of products shall not be lower than it indicated on the label.

- 5 Test Method
- 5.1 Sampling
- 5. 1. 1 Sampling principle

The sample must be representative. All kinds of factors affecting the quality of samples should be considered to prevent them from being contaminated by exogenous factors, moisture absorption, being spoiled and bacterial growth.

5. 1. 2 Sampling tools and method

Use a spoon to take out the sample randomly three times, i.e. at beginning, in middle and at last.

- 5. 2 Sensory inspection
- 5. 2. 1 Put 10g sample on the bright place, observe its color and appearance by eyes, smell its odor by nose.
- 5. 2. 2 Disssolve 2g sample in 100ml water, heat the solution and observe its solubility.
- 5. 3 Detection for loss on drying
- 5. 3. 1 Detection criteria

This test shall comply with the WS 232-2002 common criteria for biosafety in microbiology and biomedical laboratories.

- 5. 3. 2 Instrument and equipment
 - a) Electric thermostatic drying oven (Temperature 105°C)
 - b) Weighting bottle (5cm)
 - c) Dryer (Contain anhydrous calcium chloride)
 - d) Electronic scales (The sensitivity is 0.001g)
 - e) Desiccant
- 5. 3. 3 Test method

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Mix the sample evenly (if it has large particle, it should be quickly mashed into smaller particles below 2mm). Weight precisely 1g powder and put it into the flat weighting bottle dried at 105° C to constant weight, count the loss on drying of powder according to the weight loss and sample volume. The sample shall be spread flat in the weighting bottle with the thickness no more than 5mm. The cap shall be removed and place it next to the weighting bottle, or the cap is half opened, before putting the bottle into the drier. The cap must be put back on the bottle before taking it out. After drying, the powder shall be taken out and place it cool to room temperature, and then weight it.

- 5.4 Detection of residue
- 5. 4. 1 Detection criteria
 - This test shall comply with the provision of Manual of NewTechnicalStandards and Procedures for Food Hygiene Inspection (2004).
- 5. 4. 2 Equipment
 - a) Crucible
 - b) Electronic scales (The sensitivity is 0.001g)
 - c) Resistance furnace
 - d) Drier
- 5. 4. 3 Test method and result calculation

Put powder 2g into blazed crucible with constant weight, weight precisely. Heat slowly until fully carbonized and cool to room temperature; complete ashing is made at 700-800 $^{\circ}$ C, move it to a dryer and cool to room temperature, weight precisely, then heat in 700-800 $^{\circ}$ C to constant weight, get it after precisely weighting.

- 5. 5 Detection for total nitrogen
- 5. 5. 1 Detection criteria
 - This test shall comply with the provision of Manual of NewTechnicalStandards and Procedures for Food Hygiene Inspection (2004).=

5.5.2 Reagent

- a) Potassium sulfate or anhydrous sodium sulfate
- b) Copper sulfate powder
- c) sulfuric acid
- d) sodium hydroxide 40%
- e) boric acid solution 2%
- f) zinc granule
- g) bromocresol green
- 5.5.3 Equipment
 - a) Kjeldahl flask 500ml
 - b) Filter cup
 - c) Nitrogen bulb
 - d) Conical flask 500ml
 - e) Condenser pipe
- 5. 5. 4 Test method

Take some sample powder appropriate (about 25-30mg nitrogen), weight precise, place it to 500ml dried Kjeldahl flask, then add 10g potassium sulfate (or anhydrous sodium sulfate) and 0.5g copper

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sulfate powder, and add slowly 20ml sulfuric acid along the wall of the bottle. Put a small funnel in the mouth of the Kjeldner flask and make the flask stand at an Angle of 45. Heat the flask slowly with the fire to keep the temperature of the solution below the boiling point. After the boiling stops and the solution becomes clear green, heat it for another 30 minutes and cool it. Add 250ml water slowly along the bottle wall, vibrate to mix, after cooling it then add 750ml 40% sodium hydroxide solution, pay attention to make the solution flow along the wall to the bottom, form a liquid layer, add several zinc particles, connect the Kjeldner flask with the condensing tube with nitrogen ball. Add another 50ml 20% boric acid solution to 500ml conical flask and add 10 drops of methyl red-bromocresol green mix solution. Insert the bottom of condensing tube below the liquid level of boric acid solution, gently swing the Kjeldner flask to evenly mix the solution. After heating and distilling, the tip of condensing tube is taken out of the liquid level until the total volume of receiving liquid is about 250ml. Rinse the tip by steam for 1 minute, then stop distillation after rinsing it with water. The distillate is titrated with sulfuric acid titrant (0.05mol/L) until the solution changed from blue-green to grayish-purple, and the titrated results are corrected by blank test.

5. 5. 5 Test result

Each 1ml sulfuric acid titrant (0.05mol/L) equals 1.401mg N

- 5. 6 Detection of Amino Nitrogen
- 5. 6. 1 Detection Criteria

This test shall comply with the provision of Pharmacopoeia of the People's Republic of China (2015 Edition) three.

- 5. 6. 2 Reagents and equipments
 - a) Volumetric flask 100ml
 - b) Beaker 200ml
 - c) Magnetic stirrers
 - d) Sodium Hydroxide Standard Solution 0.05N
 - e) Formaldehyde solution 36%
 - f) Microburette 10ml
 - g) Acidmeter

5. 6. 3 Test method

Put 5.0ml sample to 100ml volumetric flask, add water to the scale of it, after mixing absorb 20.0ml solution into a 200ml beaker and add 60ml water. Mix the solution with magnetic stirrer then titrate with 0.05N sodium hydroxide standard solution to pH=8.2 indicated by acidmeter.

Add 10ml formaldehyde solution, after mixing titrate with 0.05N sodium hydroxide standard solution to pH=9.2, note the number of milliliters of sodium hydroxide standard solution consumed.

At same time, take 80ml water and titrate with 0.05N sodium hydroxide standard solution to pH=8.2, add 10.0 ml formaldehyde solution, then titrate with 0.05N sodium hydroxide standard solution to pH=9.2, and the titrated results are corrected by blank test. Note the number of milliliters of sodium hydroxide standard solution consumed.

5. 6. 4 Calculation

 $(V_1-V_2) \times N \times 0.014$

- X -the content of amino nitrogen in sample, g/100ml
- V_1 the volume of sodium hydroxide standard solution consumed after adding formaldehyde to sample diluent, ml
- V₂ the volume of sodium hydroxide standard solution consumed after adding formaldehyde to blank test, ml
- V_3 consume valume of sample diluent, ml
- N Equivalent concentration of sodium hydroxide standard solution
- 0.014 Grammage of nitrogen equaled with 1ml 1N sodium hydroxide standard solution
- 5. 7 Determination of net content
- 5. 7. 1 Instrument

Balance (the sensitivity is1.0g)

5.7.2 Test method and calculation result

Weigh 5 individual portion pack goods by sensitivity balance 1.0g, deduct the quality of packing, the result is net content. Use average value.

- 6 Inspection Regulation
- 6. 1 Inspection method

Product inspection shall use the way of delivery test.

- 6. 2 Delivery test
- 6. 2. 1 Product shall be inspected batch by batch before it is delivered. Product shall be allowed to leave the factory after it passes the quality inspection.
- 6.2.2 The same batch product produced constitute a inspection batch. Each batch is sampled 300g (seperate three times, beginning, in middle and at last, 100g each time)
- 6. 2. 3 All items of this standard are the delivery inspection items.
- 6.3 Determination

All items of delivery inspection of product are qulified, the product of this batch are determinated qulified. Any one of item is not qualified, double sampling is allowed for reinspection. The reinspection is not qualified, the product of this batch is determinated unqualified.

- 7 Labeling, packaging, transportation and storage
- 7. 1 Labeling

The product label shall indicate the follow content, Medium Culture Materials, product name, product English name, product type, company brand, the name, address of factory, instruction, executive standard, Executive Standard number, Batch Number, Certificate, net content.

7.2 Packaging

7. 2. 1

Product shall use non-toxic plastic bottle or food package as the inner packaging. The package materials shall comply with the regulation GB9687-1988. The packaging specification is 250g/bottle, 10kg/bag or 25kg/bag.

The packaging requirements are:

a) For packing with plastic bottle, the seal must be waxed and sealed tightly with a non-toxic plastic bag

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b) For packing with food package, the product must be vacuumized and sealed with capper, and then covered with food package.

- 7. 2. 2 Exterior packaging is #650 carboard. The labels must be affixed to the carboards(see 7.1 for label contents). After the carboard is sealed, it should be marked with "Handle with care and store in a cool and dry place".
- 7.3 Transportation

The product of this standard shall not be mixed loading with contaminated substances in transportation and storage, and shall be protected from rain and moisture during transportation.

7.4 Storage

The product of this standard shall be storaged in a cool and dry place.

7.5 Expiration date

Under the above conditions of packaging, transportation and storage, the expiration date of the product is three years.