GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

ICS 67.060

B 22

GB/T 7416-2008

Replacing GB/T 7416-2000

Malting barley

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Issued on: June 25, 2008 **Implemented on: June 01, 2009**

Issued by: General Administration of Quality Supervision, Inspection and Quarantine;
Standardization Administration of the People's Republic of China.

Table of Contents

Malting barley

1 Scope

This Standard specifies the terms and definitions, product classification, requirements, analysis methods, inspection rules, marking, packaging, transportation and storage of malting barley.

This Standard applies to the acquisition, inspection and sale of barley specially used for beer brewing.

2 Normative references

The terms in the following documents become the terms of this Standard by reference to this Standard. For dated references, all subsequent amendments (not including errata content) or revisions do not apply to this standard. However, parties to agreements that are based on this Standard are encouraged to study whether the latest versions of these documents can be used. For undated references, the latest edition applies to this Standard.

GB/T 191, Packaging - Pictorial marking for handling of goods

GB/T 601, Chemical reagent - Preparations of reference titration solutions

GB/T 603, Chemical reagent - Preparations of reagent solutions for use in test methods (GB/T 603-2002, neq ISO 6353-1:1982)

GB 2715, Hygienic standard for grains

GB/T 5491, Inspection of grain and oilseeds - Methods for sampling and sample reduction

GB/T 6682, Water for analytical laboratory use - Specification and test methods (GB/T 6682-1992, neq ISO 3696:1987)

3 Terms and definitions

The following terms and definitions are applicable to this Standard.

3.1

malting barley

Express the results obtained to integers.

6.6.2.4 Precision

The absolute difference of two independent test results under repeatability cannot exceed 2% of the arithmetic mean value.

6.7 Protein

6.7.1 Principle

Under the action of a catalyst, use sulfuric acid to decompose the sample, so that the nitrogen in the organic compound is converted into ammonia, and the distilled ammonia is absorbed by the boric acid solution; determine the nitrogen content by the acid-base titration method.

6.7.2 Reagents and solutions

6.7.2.1 Ammonia-free water: prepared according to GB/T 603.

6.7.2.2 Concentrated sulfuric acid: 95% ~ 98%.

6.7.2.3 Sodium hydroxide solution (400 g/L): Weigh 400 g of sodium hydroxide and dissolve it in 1 L of ammonia-free water; let stand. Pipette the supernatant into a bottle with a rubber stopper.

6.7.2.4 Boric acid solution (20 g/L): Weigh 20 g of boric acid, dissolve it in water, and dilute to 1 L.

6.7.2.5 Hydrochloric acid standard titration solution [c (HCl) = 0.1 mol/L]: prepared and calibrated according to GB/T 601.

6.7.2.6 Mixed catalyst: Mix potassium sulfate (K_2SO_4) and copper sulfate ($CuSO_4 \cdot 5H_2O$) in a ratio of 10+1, and grind finely.

6.7.2.7 Bromocresol green indicator solution (1 g/L): prepared according to GB/T 603.

6.7.2.8 Methyl red indicator solution (1g/L): prepared according to GB/T 603.

6.7.2.9 Bromocresol green mixed indicator solution: Pipette bromocresol green ethanol solution and methyl red ethanol solution in a ratio of 10+4, and mix them evenly.

6.7.3 Apparatus

6.7.3.1 Kjeldahl apparatus: self-assembled instrument or complete set of instruments.

6.7.3.2 Balance: sensitivity 0.1 mg.

6.7.3.3 Acid burette: 50 mL.

V_2 – volume of hydrochloric acid standard titration solution consumed during titration of test pieces, in milliliters (mL);

V_1 – volume of hydrochloric acid standard titration solution consumed during blank titration, in milliliters (mL);

c – concentration of the hydrochloric acid standard titration solution, in moles per liter (mol/L);

14 – value of the molar mass of nitrogen, in grams per mole (g/mol) [M (N) = 14];

m – mass of the weighed test pieces, in grams (g);

X_1 – mass fraction of water content of test pieces, %;

6.25 – conversion factor of nitrogen and protein.

Express the results obtained to one decimal place.

6.7.6 Precision

The absolute difference of two independent test results obtained under repeatability cannot exceed 4% of the arithmetic mean value.

6.8 Plump grains and thin grains

6.8.1 Principle

The barley sample is sieved by kernel size in a vibrating three-layer sieve deck with different hole sizes.

6.8.2 Apparatus

6.8.2.1 Balance: sensitivity 0.1 g.

6.8.2.2 Separator: Driven by the motor through the crankshaft, it is equipped with 3 layers of sieve plates with a vertical distance of 12 mm ~ 25 mm, and has a cover and a chassis. The total height of the whole machine is 80 mm ~ 100 mm.

The separator shall meet the following requirements:

Sieve plate material: made of hard brass with a thickness of 1.3 mm ± 0.1 mm, with strip holes on it, and a processing tolerance of 0.03 mm.

Sieve plate size: 43 cm in length, 15 cm in width.

Sieve hole size: The upper part is 25 mm in length, and the lower part is 22 mm in length. Width: 2.8 mm for sieve I, 2.5 mm for sieve II, and 2.2 mm for sieve III.

7.3.1 During acceptance, the corresponding quality inspection department is responsible for inspecting batch by batch according to the provisions of this Standard.

7.3.2 The acceptance inspection items include: net content, sensory requirements, foreign materials, damage rate, water content, thousand kernels weight, 3-day germination rate, 5-day germination rate, protein, plump grains, and thin grains.

7.4 Determination rules

7.4.1 Take specimens according to Table 4, and check the packaging and net content first. Where the inspection result reaches the rejection number, judge the whole batch of products as unqualified.

7.4.2 Among the physical and chemical indicators, water content and 5-day germination rate are the main indicators of the quality grade. When other indicators are of the same grade, but one of the indicators of water content or 5-day germination rate is not of this grade, the grade of this indicator shall prevail.

7.4.3 Among the physical and chemical indicators, if all other indicators are of the same grade, and only one indicator (except water content and 5-day germination rate) is lower than this grade, it will not be degraded. But if the indicator is lower than the next grade, it will be downgraded to the next one.

7.4.4 Among the physical and chemical indicators, when all other indicators are of the same grade, but two indicators (except water content and 5-day germination rate) are lower than this grade, it will be downgraded to the next one.

8 Marking, packaging, transportation and storage

8.1 Marking

8.1.1 When the malting barley is transported to the grain depot or a specified place, the place of origin, variety name, harvest time, purchase date, category and grade shall be marked.

8.1.2 The products sold shall have a quality certificate, and shall be indicated with the name and address of the manufacturer, product name and variety, batch number, net weight, and the implementation standard code.

8.1.3 The pictorial marking for storage and transportation shall comply with the relevant provisions of GB/T 191.

8.2 Packaging

8.2.1 No matter which kind of packaging is used; different varieties and different origins must not be mixed into the warehouse.

Appendix A

(Informative)

Analysis method of self-controlled technical indicators of enterprises

A.1 Determination of mold count in barley

A.1.1 Principle

By rinsing with sterile water, wash the microorganisms on the surface of the barley in water; then, cultivate on the medium; judge the degree of mold contamination on the surface of the barley according to the number of colonies.

A.1.2 Reagents and solutions

Rose Bengal medium (31.6 g/L): Weigh 31.6 g of rose Bengal medium; add 1 000 mL of water to dissolve; aliquot; autoclave at 121 °C for 20 min for later use.

A.1.3 Apparatus

A.1.3.1 Shaker: Rotating speed 180 r/min ~ 200 r/min.

A.1.3.2 Erlenmeyer flask: 300 mL.

A.1.3.3 Petri dish: 10 cm in diameter.

A.1.3.4 Pipette: 0.2 mL.

A.1.4 Analysis steps

A.1.4.1 Weigh 10 g (accurate to 0.02 g) of kernel test pieces and pour them into a triangular flask filled with 90 mL of sterile water; tighten the cotton plug; place it on the shaker; shake at 30 °C at a speed of 180 r/min ~ 200 r/min for 30 min.

A.1.4.2 Melt the rose Bengal medium; pour it into a plate under aseptic conditions; cool it to solid before use.

A.1.4.3 Under sterile conditions, use a sterilized pipette to draw 0.2 mL of solution in A.1.4.1 and apply it on the plate (3 plates for each test piece in parallel); invert the plate; incubate at 25 °C for 7 days.

A.1.4.4 Count the number of colonies on the plate.

A.2 Identification of barley varieties

A.2.1 Gel electrophoresis

A.2.1.1 Principle

Identify barley varieties by polyacrylamide gel electrophoresis (PAGE), and use slab gel electrophoresis to separate the alcohol soluble protein of barley.

A.2.1.2 Reagents and solutions

A.2.1.2.1 Extract: Weigh 18 g of urea and 0.01 g of methyl green; dissolve in water; then, add 1 mL of 2-sulfhydryl ethanol and 20 mL of 2-chloroethanol; then, use water to dilute to 100 mL.

A.2.1.2.2 Ferrous sulfate solution (5 g/L): prepared according to GB/T 603.

A.2.1.2.3 Gel stock solution: Weigh 115.3 g of acrylamide, 4.6 g of methylenebisacrylamide, 69.2 g of urea, 1.2 g of glycine, 1.2 g of ascorbic acid; dissolve in water; add 3 mL of ferrous sulfate solution (newly prepared), 23 mL of glacial acetic acid; mix well, and dilute to 1 L. After suction filtration, put it into a brown bottle; store it at 4 °C; use it within the same month.

A.2.1.2.4 Hydrogen peroxide solution: Absorb 2 mL of 30% hydrogen peroxide; use water to dilute to 100 mL.

A.2.1.2.5 Electrode buffer solution: Weigh 2 g of glycine; dissolve it in water; add 20 mL of glacial acetic acid; add water to 5 L.

A.2.1.2.6 Trichloroacetic acid solution (10 g/L): Weigh 10 g of trichloroacetic acid; dissolve it in water; dilute to 100 mL.

A.2.1.2.7 Coomassie brilliant blue solution (10 g/L): Weigh 1 g of Coomassie brilliant blue; dissolve it in 95% ethanol; dilute to 100 mL.

A.2.1.2.8 Staining solution: Take 20 mL of trichloroacetic acid solution; add 1 mL of Coomassie blue solution; mix well and set aside.

A.2.1.3 Apparatus

A.2.1.3.1 Vertical plate gel electrophoresis apparatus.

A.2.1.3.2 Analytical balance: sensitivity 0.1 mg.

A.2.1.3.3 Centrifuge: rotating speed 5 000 r/min, centrifuge tube Φ9 mm × 35 mm;

A.2.1.4 Analysis steps

A.2.1.4.1 Number of samples: Take 100 kernels of barley for this determination.

A.2.1.4.2 Extraction of hordein: Grind each kernel of barley separately and put it into a centrifuge tube; draw 0.4 mL of extract and mix; soak for at least 16 h; put the

A.2.2.2.2 EDTA solution (0.5 mol/L, pH = 8.0): Weigh 186.1 g of ethylene diamine tetraacetic acid (EDTA, $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$); add it to 800 mL of water; stir on a magnetic stirrer; use sodium hydroxide to adjust the pH value of the solution to 8.0 (approximately 20 g of NaOH granules are needed); then, dilute to 1 L; autoclave after aliquoting.

A.2.2.2.3 DNA extract: Weigh 46.75 g of sodium chloride and 20 g of cetyltrimethylamine bromide (CTAB); add 800 mL of deionized water; shake the container to dissolve the solute completely. Then, add 50 mL of Tris-hydrochloric acid solution (A.2.2.2.2) and 20 mL of EDTA solution (A.2.2.2.2); use water to dilute to 1 L; autoclave after aliquoting.

A.2.2.2.4 Trichloromethane-isoamyl alcohol solution (24+1): Measure 240 mL of trichloromethane and 10 mL of isoamyl alcohol and mix well.

A.2.2.2.5 Ribonuclease A (RNaseA, 10 mg/mL): purchased from a biochemical reagent company.

A.2.2.2.6 PCR amplification reagents: purchased from biochemical reagent companies [PCR amplification reagents include: Taq DNA polymerase, dNTP, magnesium chloride ($MgCl_2$), PCR buffer (containing $MgCl_2$), primers].

A.2.2.2.7 Electrophoresis buffer I (10×TBE): Weigh 108 g of Tris base, 55 g of boric acid and 7.44 g of EDTA and mix; use redistilled water to dissolve; then, dilute to 1 L.

A.2.2.2.8 Electrophoresis buffer II (50×TAE): Weigh 242 g of Tris base and 37.2 g of EDTA and mix; add 57.1 mL of glacial acetic acid; use redistilled water to dissolve; dilute to 1 L.

A.2.2.2.9 TE buffer solution: Add 10 mL of Tris-hydrochloric acid solution (A.2.2.2.1) and 2 mL of EDTA solution (A.2.2.2.2) in 800 mL of water in turn; add water to fix volume to 1 L; aliquot and autoclave.

A.2.2.2.10 Sterile water: Take 100 mL ~ 200 mL of redistilled water, and sterilize at 121 °C for 20 min.

A.2.2.2.11 Denaturing sample loading buffer: Weigh 10 g of sucrose, 20 mg of bromophenol blue, 20 mg of xylene cyanol; dissolve in 90 mL of deionized formamide; use redistilled water to dilute to 100 mL.

A.2.2.2.12 Stationary liquid (10%): Measure 100 mL of glacial acetic acid; add 900 mL of redistilled water; mix well.

A.2.2.2.13 Sodium thiosulfate solution (10%): Weigh 10 g of sodium thiosulfate; add 100 mL redistilled water to dissolve.

A.2.2.2.14 Gel solution (6%): Weigh 60 g of acrylamide, 3.1 g of methylene bisacrylamide, 420 g of urea and draw 50 mL of electrophoresis buffer I (A.2.2.2.7); mix well; use redistilled water to dissolve; dilute to 1 L.

A.2.2.2.15 Gel staining solution: Weigh 1 g of silver nitrate; add 1 000 mL of redistilled water; then, add 1.5 mL of formaldehyde; mix well.

A.2.2.2.16 Gel developer: Weigh 30 g of anhydrous sodium carbonate; add 1 000 mL of redistilled water to dissolve; then, add 0.2 mL of sodium thiosulfate (A.2.2.2.13) and 1.5 mL of formaldehyde; mix well.

A.2.2.2.17 Agarose gel (0.8%): Weigh 0.8 g of agarose; add 100 mL of 1×TAE electrophoresis buffer II; heat to dissolve.

A.2.2.2.18 Ethanol (70%): Absorb 700 mL of absolute ethanol; add water to dilute to 1 L.

A.2.2.3 Apparatus

A.2.2.3.1 PCR apparatus: sensitive and accurate response to temperature gradient changes.

A.2.2.3.2 Constant voltage electrophoresis apparatus: voltage 10 V ~ 3 000 V, current 2 mA ~ 200 mA, power 5 W ~ 200 W.

A.2.2.3.3 Centrifuge: low temperature to below 4 °C, rotation speed 10 000 r/min.

A.2.2.3.4 Constant temperature water bath: temperature control accuracy ±0.1 °C.

A.2.2.3.5 Micropipette: accurate to 0.1 mL.

A.2.2.3.6 Gel imager: can be connected to a computer to take pictures.

A.2.2.3.7 Electrophoresis tank: vertical electrophoresis tank and horizontal electrophoresis tank.

A.2.2.3.8 Rotary horizontal decolorization shaker: speed 1 000 r/min ~ 10 000 r/min.

A.2.2.3.9 X-ray lamp.

A.2.2.3.10 Glass plate.

A.2.2.3.11 Centrifuge tube: 1 mL ~ 2 mL.

A.2.2.3.12 PCR thin-walled tubes.

A.2.2.3.13 Analytical balance: sensitivity 0.1 mg.

A.2.2.4 Analysis steps

(1) Denaturation at 94 °C for 30 s; (2) Annealing at 55 °C for 40 s; (3) Extension at 72 °C for 40 s. Finally, complete the amplification program by incubating at 72 °C for 10 min; take out the amplified product and place in a refrigerator at 4 °C for later use.

A.2.2.4.3 Preparation of polyacrylamide gel plates

a) First rinse the glass plate and ear plate with tap water.

b) After distinguishing the front and back of the two glass plates, wipe them with ethanol, respectively.

c) After the ethanol volatilizes, apply affinity silane evenly on one side of the plate twice; apply peeling silane evenly on the ear plate twice (perform this operation under a fume hood, be careful of corrosion).

d) After the above steps are completed, place the two bands on both sides of the plate (the side coated with the affinity silane) respectively; cover the ear plate (the side coated with the peeling silane) on the plate; tighten it with clips.

e) Slowly pour the gel stock solution (A.2.2.2.14) along the gap between the two plates (on the side of the ear plate); avoid air bubbles during the filling process to avoid affecting the experiment (60 mL of gel stock solution + 200 μL of 10% ammonium persulfate + 100 μL of TEMED).

f) After the gel is poured, insert the comb (the end without teeth) into the gel surface, and the insertion depth shall be appropriate.

g) Place the gel at room temperature for 1 h ~ 2 h (determine the gel time according to the temperature).

A.2.2.4.4 Pre-electrophoresis

a) After gelation and fixation, pull out the comb, place the plate side outward, and the ear plate side inward; put it in the vertical electrophoresis tank; tighten it with iron clips at both ends.

b) Dilute the electrophoresis buffer I (A.2.2.2.7) to 0.5×TBE; pour it into the upper and lower tanks of the vertical electrophoresis tank; blow off the broken gel at the sample end of the gel plate.

c) After connecting the power cord of the electrophoresis tank to the electrophoresis apparatus, turn on the power. Perform constant power electrophoresis at 70 W for 30 min.

A.2.2.4.5 Loading and running electrophoresis

a) Turn off the power after 30 min of pre-electrophoresis; insert the comb (the end with teeth) into the gel plate to a suitable depth.

b) Add the PCR amplification product to the denaturing sample loading buffer; perform denature in the PCR apparatus at 94 °C for 5 min; quickly transfer to ice water to cool.

c) Take 3 μL ~ 5 μL of denatured amplification products and add them along the comb holes; turn on the power supply; perform constant power electrophoresis at 70 W for 45 minutes.

A.2.2.4.6 Silver staining development

a) After electrophoresis, separate the glass plate; put the plate (glass plate with gel) into the elution box containing the stationary liquid (A.2.2.2.12); shake it on the rotary horizontal shaker for 20 min ~ 30 min; fix it; decolor.

b) After decolorization, use redistilled water to wash for 2 ~ 3 times, 3 min/time ~ 5 min/time.

c) Use the gel staining solution (A.2.2.2.15) to stain for 20 min ~ 30 min.

d) Use redistilled water to wash quickly for 8 s ~ 10 s.

e) Use gel developer (A.2.2.2.16) to develop until a clear band is visible.

f) Use 10% glacial acetic acid to fix for 3 min ~ 5 min.

g) Use re-distilled water to wash for 3 min ~ 5 min, and air-dry at room temperature.

A.2.2.4.7 Display of results

Put the air-dried glass plate on the X-ray light box; observe and compare it with the genetic map of the original pure variety of the known barley variety; record it in detail.

A.3 Determination of water sensitivity

A.3.1 Principle

Take two groups of test pieces; add 4 mL and 8 mL of water respectively; keep warm and germinate. The difference in the percentage of sprouted kernels between the two groups is the water sensitivity.

A.3.2 Apparatus

A.3.2.1 Petri dish: 10 cm in diameter.

A.3.2.2 Graduated pipette: graduation value 0.1 mL.

A.3.3 Analysis steps

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