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Replacing GB/T 12143.1~12143.3-1989, GB/T 12143.4~12143.5-1992, GB/T 16771-1997

General Analytical Methods for Beverage

饮料通用分析方法

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Foreword

This Standard replaced the following standards including GB/T 12143.1-1989 *Determination Method for Soluble Solids Content in Soft Drinks – Refractometric*; GB/T 12143.2-1989 *Determination Method for Amino Nitrogen Content in Fruit (Vegetable) Juice Products – Formol Number*; GB/T 12143.3-1989 *Determination Method for L-Ascorbic Acid in Fruit and Vegetable Juice Beverages – Ethyl Ether Extraction Method*; GB/T 12143.4-1992 *Method for Determination of Carbon Dioxide in Carbonated Soft Drink*; GB/T 12143.5-1992 *Method for Determination of Ethanol in Concentrated Fruit Juice*; GB/T 16771-1997 *Determination of Juice Content in Orange, Mandarine, Tangerine Juice and Their Drinks*.

Compared with GB/T 12143.1 ~ 12143.3-1989, GB/T 12143.4 ~ 12143.5-1992, GB/T 16771-1997, this Standard mainly has the following change: combine the following 6 standards, including GB/T 12143.1~12143.3-1989, GB/T 12143.4~12143.5-1992, GB/T 16771-1997, into one standard.

The Appendix C, D, E, F and G of this Standard are normative; while the Appendix A and B are informative.

This Standard was proposed by National Technical Committee on Food Industry of Standardization Administration of China.

This Standard shall be under the jurisdiction of National Technical Committee for Standardization of Beverage.

Chief drafting organizations of “Determination Method for Soluble Solids Content in Soft Drinks (Refractometric)”, “Determination Method for Amino Nitrogen Content in Fruit (Vegetable) Juice Products (Formol Number)”, “Method for Determination of Carbon Dioxide in Carbonated Soft Drink (Distillation and Titration Method)”, in this Standard: China National Research Institute of Food Fermentation Industries. Chief drafting staff of the above contents in this Standard: Xu Qingqu, and Gong Lingdi.

Chief drafting organizations of “Determination Method for L-Ascorbic Acid in Fruit and Vegetable Juice Beverages (Ethyl Ether Extraction Method)”, “Method for Determination of Ethanol in Concentrated Fruit Juice”, in this Standard: China National Research Institute of Food Fermentation Industries. Chief drafting staff of the above contents in this Standard: Gong Lingdi, and Xu Qingqu.

Responsibly drafting organizations of “Determination of Juice Content in Orange, Mandarine, Tangerine Juice and Their Drinks”, in this Standard: National Technical Committee on Food Industry of Standardization Administration of China, China National Research Institute of Food Fermentation Industries, China Agricultural University, Zhejiang Provincial Light Industry Research Institute, Sichuan Provincial Light Industry Research Institute, Hunan Provincial Food Quality Supervision and

General Analytical Methods for Beverage

1 Scope

This Standard specifies the general rules and specific analytical methods of the general analytical methods for beverage.

This Standard is applicable to the analysis of specific components in the beverage.

2 Normative References

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 601 Chemical Reagent - Preparations of Standard Volumetric Solutions

GB/T 6682 Water for Analytical Laboratory Use – Specification and Test Methods (GB/T 6682-2008, ISO 3696:1987, MOD)

GB 10789 General Standard for Beverage

3 General Rules

3.1 The terminologies and measuring units used in this Standard shall conform to the provisions of relevant national standards.

3.2 The “instrument” in this Standard indicates the instrument required for analysis; general laboratory used instrument and equipment are not listed item by item.

3.3 The water used in this Standard shall, if not indicated other requirements, conform to the specification of Class-3 above (including Class-3) water stipulated in GB/T 6682. The reagents used shall, if not indicated other specifications, refer to the analytically pure.

3.4 The “solution” in this Standard shall, unless otherwise is specified, refer to aqueous solution.

that the carbon dioxide can be excluded as much as possible. After cooling off, add water to the original mass; mix thoroughly, and use for testing.

5.5.4 Fruit and vegetable juice solid beverage

Take 125g (accurate to 0.001g) of specimen; dissolve into distilled water; transfer it totally into 250mL volumetric flask; use distilled water to dilute to the scale. Mix thoroughly, use for testing.

5.6 Analytical procedures

5.6.1 Turn on the acidometer, after pre-heating for 30min; use pH 6.8 buffer solution to correct the acidometer.

5.6.2 Absorb appropriate amount of sample solution (amino nitrogen content is 1mg ~5mg) into the beaker; add 5 drops of 30% hydrogen peroxide. Place the beaker onto the electromagnetic stirrer; the electrode is inserted into the proper position of the specimen in the beaker. If necessary, add appropriate amount of distilled water.

5.6.3 Start the electromagnetic stirrer; use 0.1mol/L sodium hydroxide standard solution slowly to neutralize the organic acid in the specimen. When pH reaches about 7.5, use 0.05mol/L sodium hydroxide standard titration solution to adjust pH to be 8.1; keep 1min unchanged. Then slowly add 10mL ~ 15 mL neutral formaldehyde solution. After 1min, use 0.05mol/L sodium hydroxide standard titration solution to adjust pH to be 8.1. Record the consumed milliliters of 0.05mol/L sodium hydroxide standard titration solution.

5.7 Result calculation

The amino nitrogen in the specimen shall be calculated as per Formula (1):

$$X = \frac{c \times V \times K \times 14}{m} \times 100 \quad \dots\dots\dots (1)$$

Where:

X – the number of milligrams of amino nitrogen per 100g (100mL) specimen; in mg/100g or mg/100mL;

c – the concentration of sodium hydroxide standard titration solution; in mol/L;

V – after adding neutral formaldehyde solution, the consumed volume of 0.05mol/L sodium hydroxide standard titration solution by titrating specimen, in mL;

K – dilution times;

14 – the number of milligrams of nitrogen equivalent to 1mL 1mol/L sodium hydroxide standard titration solution;

6.3.6 Iodine standard titration solution [$c(\frac{1}{2}I_2) = 0.1 \text{ mol/L}$]: when using, dilute $0.1 \text{ mol/L}(\frac{1}{2}I_2)$

iodine standard titration solution to $V_{25 \text{ mL}} \rightarrow V_{250 \text{ mL}}$; the concentration is expressed by C_1 .

6.3.7 0.88mg/mL ascorbic acid standard solution: take 0.22g of ascorbic acid; dissolve into the oxalic acid solution (6.3.4), and dilute to 250mL.

Calibration: absorb 20.00mL of ascorbic acid standard solution; add 1mL starch indicator solution (6.3.10); titrate with 0.01mol/L iodine standard solution (6.3.6) till it turns to slight blue.

L-ascorbic acid concentration shall be calculated as per Formula (2):

$$c_2 = \frac{c_1 \times V_1}{20} \times 88 \dots\dots\dots (2)$$

Where:

c_2 - L-ascorbic acid concentration; in mg/mL;

c_1 – concentration of iodine standard titration solution; in mol/L;

V_1 – when calibrating, the used volume of iodine standard titration solution; in mL;

88 – the number of milligrams of L-ascorbic acid equivalent to $1 \text{ mL } 0.1 \text{ mol/L}(\frac{1}{2}I_2)$ iodine standard titration solution.

6.3.8 0.088mg/mL L-ascorbic acid standard solution: absorb 25.00mL of 0.88mg/ml L-ascorbic acid standard solution (6.3.7); dilute with oxalic acid solution (6.3.4) to $V_{25 \text{ mL}} \rightarrow V_{250 \text{ mL}}$.

NOTE: L-ascorbic acid solution in 6.3.7 and 6.3.8 shall be prepared when using.

6.3.9 2,6-dichloroindophenol standard titration solution: take 200mg of 2,6-dichloroindophenol; wet with small amount of hot double distilled water; then slowly add hot double distilled water; mix and dissolve, filter. After cooling off, use double distilled water to dilute the filtrate to 1L; store into the refrigerator; Calibrate at least once per week.

Calibration: absorb 10.00mL of L-ascorbic acid standard solution (6.3.8); and place into 50mL colorimetric tube; calibrate the titration of 2,6-dichloroindophenol solution as per measured sample procedures.

The titration of 2,6-dichloroindophenol solution shall be calculated as per Formula (3):

6.5.4 Fruit and vegetable juice carbonated beverage

Firstly, shake the sample until it is substantially free from bubbles; prepare as per 6.5.3.

6.5.5 Solid beverage

Take the representative sample containing 4mg~10mg of ascorbic acid (accurate to 0.001g); use 2% oxalic acid solution to dissolve and dilute to 200mL, mix evenly for testing.

6.5.6 Extraction of ethyl ether

For the high-degree emulsified sample, the sample liquid color is darker, and is easy to be extracted by ethyl ether, after sampling, place into the separatory funnel. Add 30mL of ethyl ether; sufficiently shake rather than emulsifying. After layering, the lower-layer sample liquid shall be placed into 200mL volumetric flask; add 20mL of 2% oxalic acid solution into the separatory funnel. Appropriately shake, after layering, place the lower-layer aqueous solution into the above 200mL volumetric flask. Repeat such operation for 4 times; place each lower-layer aqueous solution into the 200mL volumetric flask; then use 2% oxalic acid to dilute to the scale.

6.5.7 Preparation of blank test solution

Take the same sample (accurate to 0.001g) as the sampling amount determined during the preparing period of the test solution; place into 250mL conical flask; add 20mL of 10% copper sulfate solution; add water so that the total volume to be 100mL; place onto the electric stove padded with asbestos gauze; carefully heating to boiling, and keep slightly boiling for 15min; then use the flowing water to cool off to the room temperature. Transfer the solution into the 200mL volumetric flask; use water to dilute to the scale; shake evenly for the blank testing.

6.6 Analytical procedures

6.6.1 Determination of test solution

Take 10 pieces ~ 15 pieces of 50mL colorimetric tube; add 10.00mL of solution prepared as per 6.5 into each colorimetric tube; and add 2.5mL of acetone in each tube. After standing for 3min; add 1mL of 2,6-dichloronindophenol solution (6.3.9) into the first piece of colorimetric tube; mix evenly, after precisely controlling for 40s, add 2mL of ethyl ether, shake thoroughly, stand for several minutes until the ethyl ether and aqueous solution are layered; observe the ether layer whether there is rose red color occurred. When the rose red color occurs, it indicates the tentative end-point of the measurement has been reached. If 2,6-dichloronindophenol is totally reduced by ascorbic acid, the ethyl ether layer maintains colorless; then add 1.5mL of 2,6-dichloronindophenol solution into the second piece of colorimetric tube. If red color still not appears, then add 2.0mL, 2.5mL, 3.0mL, 3.5mL, 4.0mL, 4.5mL, 5.0mL of 2,6-dichloronindophenol solution, respectively till the ethyl ether layer occurs rose red color,

of water containing no carbon dioxide and 3 drops of phenol-thymolphthalein indicator solution (7.3.5); titrate with 0.25mol/L sodium hydroxide standard titration solution (7.3.7); when it is close to the end-point, heating the solution in the conical flask to 80°C; continue to titrate till the solution turns to lavender color.

The concentration of hydrochloric acid standard titration solution shall be calculated as per Formula (6):

$$c_4 = \frac{c_3 \times V_4}{20.0} \dots\dots\dots (6)$$

Where:

c_4 – the concentration of hydrochloric acid standard titration solution; in mol/L;

c_3 – the concentration of sodium hydroxide standard titration solution; in mol/L;

V_4 – when titrating, the consumed volume of sodium hydroxide standard titration solution; in mL;

20.0 – when titrating, the taken volume of hydrochloric acid standard titration solution, in mL.

7.4 Instrument and equipment

7.4.1 Carbon dioxide distillation and absorption device (see Figure 1).

7.4.2 Desktop vacuum pump or suction pipe (Gamma).

7.4.3 Vacuum meter: measuring range of 1kPa~100kPa (0mmHg~760mmHg).

7.4.4 Refrigerator or ice-salt water bath.

7.4.5 Analytical balance: sensibility of 0.0001g.

7.5 Preparation of test solution

Place the new soft drink into ice-salt bath at 0°C below (or in the freezing chamber of the refrigerator); dip for 1h~2h; when the soft drink in the bottle is nearly to be frozen (no shaking), open the bottle cap; quickly add supernatant liquid of 50% sodium hydroxide solution (add 2.0mL~2.5mL per 100mL of soft drink); immediately cover with rubber stopper plug. Put the bottom of the bottle upward; slowly shake for several minutes, store at room temperature, and to be measured.

7.6 Analytical procedures

7.6.1 Distillation – absorption of test solution

Take 15.00mL ~ 25.00mL of the above prepared solution (carbon dioxide content is 0.06g~0.15g) into 500mL round bottom flask with support; add 3mL of 10% hydrogen peroxide solution (7.3.4) and several pieces of porous ceramic sheets; connect with the absorption tube; connect the separatory funnel tightly to the flask, no leakage. Accurately pre-add 20mL of 0.25mol/L sodium hydroxide standard titration solution (7.3.7) into the first and the second absorption tubes; place the two absorption tubes into the flask contained water; during the distillation and absorption process, the temperature shall be controlled at 25°C below. Accurately add 10mL of 0.25mol/L sodium hydroxide standard titration solution (7.3.7) and 10mL of barium chloride solution (7.3.3) into the third absorption tube. Connect the three absorption tubes in series. One end of the third absorption tube is connected to the vacuum pump, so that the entire device is sealed (see Figure 1). Open the valve connecting to the vacuum pump, slowly increase the vacuum degree, and control to be 14kPa~20kPa (100mmHg~150mmHg) till there is no bubble passing through the absorption tube. Continue to pump air, maintain the vacuum state; add 35mL of acidic phosphate solution (7.3.2) into the separatory funnel; open the piston, so that the acidic phosphate solution slowly drops into the flask (about 30mL); close the piston; shake the flask; mix the sample with acid liquid thoroughly; use the pressure regulator to control the temperature of the electric furnace; slowly heating, so that the carbon dioxide escapes slowly; the control the absorption tubes to have intermittent bubbles rise, till the first absorption tube is increased 2mL~3mL of distillate. When the upper part of the absorption tube feels warm, namely, the carbon dioxide in the flask has been escaped totally, and is absorbed by the sodium hydroxide in the absorption tube. At this time, close the junction valve between the third absorption tube and the vacuum pump; close the electric furnace; slowly open the piston of the separatory funnel, ventilate into air; so that the pressure is balanced. Combine the solution into the three absorption tubes, and wash into the 500mL conical flask; use small amount of water to wash the absorption tubes for several times; the washing liquid shall be injected into the conical flask; add 50mL of barium chloride solution (7.3.3), shake evenly, and stand for a moment.

in g/mL;

46.07 – the relative molecular mass of ethanol;

m_4 – taken mass of potassium dichromate; in g;

294.18 – the relative molecular mass of potassium dichromate.

8.3.5 Ammonium ferrous sulfate standard titration solution: take 17g of ammonium ferrous sulfate, dissolve into water; add 20mL of sulfuric acid, dilute to 1000mL; store into the brown reagent bottle; 2mL of such solution is equivalent to 1mL of potassium dichromate solution (8.3.4).

NOTE: since such solution is unstable, easy to be oxidized in the air; it shall be calibrated by potassium dichromate standard solution in the same day when it is used; when it is about to be used, transfer into the brown burette.

8.3.6 Phenanthroline iron indicator solution: take 0.659g of ferrous sulfate and 1.485g of phenanthroline; dissolve into 100mL of water.

8.3.7 Bromothymol blue indicator solution: 1%; take 1g of bromothymol blue; add 8mL of 0.2mol/L sodium hydroxide solution; use water to dissolve and dilute to 100mL.

8.4 Instrument and equipment

8.4.1 Constant temperature water bath: 100°C±1°C.

8.4.2 Distillation device (see Figure 2).

~ 0.1g/kg; the difference shall not exceed 5% of the average value.

9 Determination of Juice Content in Orange, Mandarine, Tangerine Juice and Their Drinks

9.1 Applicable range of method

This Method is applicable to judge the concentrated juice and fruit juice in orange, mandarine, tangerine; as well as the orange, mandarine, tangerine drinks with juice content no less than 2.5%.

9.2 Terms and definitions

The following terms and definitions are applicable to this Method.

9.2.1 Concentrated juice, fruit juice and its beverage

The definitions are the same as GB 10789.

9.2.2 Standard value

The reasonable value determined by mathematical statics against the soluble solids contents in the fruit juice restored by concentrated juice; and that contents in the juice of orange, mandarine, tangerine with different types, different producing areas, different harvesting periods, different processing technologies, different storing periods; as well as the distributing state of actually-measured values of 6 components.

9.2.3 Weighted value

Certain component's proportion in the overall share shall be determined according to the size of the coefficient of variation of 6 components' actually-measured values.

9.3 Principle of method

9.3.1 The method specified in this Standard measures the 6 components in the sample.

9.3.2 After rationally rounding off the ratio between the actually-measured values of 6 components and their standard values; which shall be multiplied by the corresponding rounding-off weighted value; then plus item by item to obtain the fruit juice content in the sample.

9.4 Standard values and weighted values of orange, mandarine, tangerine juice and their mixed juice

9.4.1 Standard values of soluble solids

Appendix C

(Normative)

Determination of Potassium

C.1 Principle of method

The potassium base state atoms absorb the resonance line emitted from the potassium hollow cathode lamp; the absorption intensity is proportional to the potassium concentration. Absorb the treated sample into the flame atomization system of the atomic absorption spectrophotometer to atomize the potassium ions; determine the absorbance at 766.5nm of resonance line; compared with standard serial solution, determine the potassium content in the sample. Add appropriate amount of sodium salt, and eliminate the ionization interference.

C.2 Reagents

C.2.1 Nitric acid.

C.2.2 Sulfuric acid.

C.2.3 10g/L sodium chloride solution: take 1.0g of sodium chloride, dissolve into water, and make constant volume to 100mL.

C.2.4 Nitric acid solution with volume fraction of 10%: take 1 volume of nitric acid (C.2.1); inject into 9 volumes of water.

C.2.5 Hydrochloric acid solution with volume fraction of 50%: take 1 volume of hydrochloric acid; inject into 1 volume of water.

C.2.6 Potassium standard solution: take 0.9534g of potassium chloride baking for 2h at $150^{\circ}\text{C}\pm 3^{\circ}\text{C}$, accurate to 0.0001g. Place into 50mL beaker. Add water to dissolve, transfer to 500mL volumetric flask. Add 2mL of hydrochloric acid solution (C.2.5), use water to make constant volume to the scale, shake evenly. Absorb 10.00mL of such solution into 100mL volumetric flask, use water to make constant volume to the scale, shake evenly. The potassium content of this solution is 100mg/L.

C.3 Instrument and equipment

C.3.1 Atomic absorption spectrophotometer: with potassium hollow cathode lamp.

C.3.2 Air compressor or air cylinder gas.

C.3.3 Acetylene cylinder gas.

C.3.4 Kjeldahl flask: 500mL.

Appendix D

(Normative)

Determination of Total Phosphorus

D.1 Principle of method

After the digestion of the sample, the phosphate reacts with vanadium-ammonium molybdate under acidic conditions, and turns to be yellow; measure the absorbance of the solution at the wavelength of 400nm; compared with the standard serial solution, and determine the total phosphorus content in the sample.

D.2 Reagents

D.2.1 Nitric acid.

D.2.2 Sulfuric acid.

D.2.3 Sulfuric acid solution with volume fraction of 10%: take 1 volume of sulfuric acid (D.2.2); slowly inject into 9 volumes of water.

D.2.4 Vanadium-ammonium molybdate solution: take 2.0g of ammonium molybdate, dissolve into 400mL of 50°C warm water; cool off. Take 1.0g of ammonium metavanadate, dissolve into 300mL of 50°C warm water; cool off; add 1mL of sulfuric acid (D.2.2) as stir. Slowly add ammonium molybdate solution into ammonium metavanadate solution; stir evenly; then transfer into 1000mL volumetric flask; use water to make constant volume to scale.

D.2.5 Phosphorus standard solution: take 0.4394g of potassium dihydrogen phosphate baking for 2h at 105°C±2°C, accurate to 0.0001g. Place into 50mL beaker. Add water to dissolve, transfer into 1000mL volumetric flask; use water to make constant volume to the scale; shake evenly. The phosphorus content in such solution is 100mg/L.

D.3 Instrument

D.3.1 Ultraviolet spectrophotometer.

D.3.2 Kjeldahl flask: 500mL.

D.3.3 Balance: sensibility of 10mg.

D.3.4 Analytical balance: sensibility of 0.1mg.

D.4 Preparation of test solution

F.2.8 Buffer solution: take 2.4g of tris(hydroxymethyl)methyl aminomethane and 0.035g of disodium ethylenediaminetetraacetate; use 80mL of water to dissolve. Firstly, use 4mol/L hydrochloric acid to adjust pH=7.2 or so; then use 1mol/L hydrochloric acid to adjust pH=7.0 (measured by acidometer); use water to make constant volume of 100mL.

F.2.9 Ammonia.

F.2.10 Acetone.

F.2.11 Washing solution: take 150mL of water, add 10mL of ammonia (F.2.9), 100mL of acetone (F.2.10), mix evenly.

F.3 Instrument and equipment

F.3.1 UV spectrophotometer: with quartz cuvette, optical path of 1cm.

F.3.2 Acidometer: precision of 0.1pH unit.

F.3.3 Centrifuge: speed no less than 4000r/min; centrifuge tube volume greater than 80mL.

F.3.4 Adjustable micropipette:

10μL~50μL, allowable difference: $\pm 4.8\%$;

0μL~1000μL, allowable difference: 100μL, $\pm 2.0\%$; 500μL, $\pm 1.0\%$; 1000μL, $\pm 1.0\%$.

F.3.5 Glass rod or plastic rod: self-made; diameter about 3mm; one end with hook.

F.3.6 Analytical balance: sensibility of 0.1mg.

F.3.7 Balance: sensibility of 10mg, 500mg, 1g.

F.4 Preparation of test solution

F.4.1 Fruit juice type carbonated beverage

Take 500g of sample into the 1000mL of beaker; heating and boiling; maintain for 5 min under the slightly boiling state; continue to stir. After the carbon dioxide is removed completely, cool off to the room temperature; weigh it. Use water to make up to the mass before heating, for backup.

F.4.2 Concentrated fruit juice, fruit juice, fruit juice beverage, and fruit beverage

Mix evenly for backup.

F.5 Analytical procedures

Appendix G

(Normative)

Determination of Total Flavonoids

G.1 Principle of method

The flavonoids (hesperidin, new hesperidin, etc.) in orange, mandarine, tangerine react with alkali; open-loop generates 2,6-dihydroxy-4-epoxy phenyl acetone, and anisic aldehyde; in the diethylene glycol environment, when encountering alkali, concentrate and generate yellow hesperidin chalcone; the generating amount is equivalent to the amount of hesperidin. Perform colorimetric determining of absorbance at wavelength of 420nm; after deducting the background, compared with standard series, and quantify.

G.2 Reagents

G.2.1 0.1mol/L sodium hydroxide solution

Take 4g of sodium hydroxide; add water to dissolve; make constant volume to be 1000mL.

G.2.2 4mol/L sodium hydroxide solution

Take 16g of sodium hydroxide; add water to dissolve; make constant volume to be 100mL.

G.2.3 200g/L citric acid solution

Take 20g of citric acid; add water to dissolve, make constant volume to be 100mL.

G.2.4 Diethylene glycol solution with volume fraction of 90%

Take 90mL of diglycol (also known as diethylene glycol); add 10mL of water, mix evenly for backup.

G.2.5 Reagent blank solution

Take 20mL of sodium hydroxide solution (G.2.1) into 50mL beaker; use citric acid solution (G.2.3) to adjust to pH = 6; transfer into 100mL volumetric flask; use water to make constant volume to scale; shake evenly.

G.2.6 Hesperidin standard solution

Take 0.0250g of hesperidin [hesperidin (consisting of hesperetin and 7-rhamnoglucoside); molecular formula: $C_{28}H_{34}O_{15}$; relative molecular mass: 610.6;

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