JAPANESE AGRICULTURAL STANDARD JAS
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Flavored seasoning

1 Scope

This document specifies the quality of flavored seasoning.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. The latest edition of the referenced document (including any amendments) applies.

CODEX STAN 192, *General Standard for Food Additives*

JIS K 0124, *General rules for high performance liquid chromatography*

JIS K 0557, *Water used for industrial water and wastewater analysis*

JIS P 3801, *Filter paper (for chemical analysis)*

JIS R 3505, *Volumetric glassware*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
flavoring material
powder or extract of *fushi-rui* [*katsuo-bushi* (*fushi* of bonito), etc.], boiled and dried small fishes, *kombu*, shell ligaments, dried *shiitake*, etc.

3.2
flavored seasoning
product which adds flavor and taste of *flavoring material* (3.1) while cooking; prepared by adding sorts of sugar, salt, etc. (excluding spices) to seasoning (amino acid, etc.) and *flavoring material* (3.1), drying them and forming them to powder, granules, etc.

4 Quality

4.1 Properties

The properties are as follows:

a) The flavor and taste and the color and luster shall be good, and not have an objectionable taste or odor;

b) Those in powder or granular form shall not have lumps, etc. caused by moisture absorption, with the

granules being separated, and those in other forms shall not be crumbled, with the original shape being maintained.

4.2 Sugar content and salt content

The sugar content shall be 40 % or less, when tested by the method specified in 5.2; the salt content shall be 35 % or less, when tested by the method specified in 5.3; and the total amount of the sugar content and the salt content shall be 65 % or less.

4.3 Total nitrogen content

The total nitrogen content shall be as follows, when tested by the method specified in 5.4:

a) 2,7 % or more, when powder and an extract of *katsuo-bushi* (*fushi* of bonito) and an extract of bonito are used as flavoring materials;

b) 2,5 % or more, when powder and extracts of boiled and dried sardine, boiled and dried flying fish are used as flavoring materials.

4.4 Compounded amount of starch and dextrin

The compounded amount of starch and dextrin shall be 2 % or less as the weight proportion of starch and dextrin used as the raw materials in the ingredients and the additives.

4.5 Composition percentage of flavoring materials

The composition percentage of powder and an extract of flavoring materials, calculated by the following formula, shall be 8,3 % or more.

$$\text{Composition percentage of flavoring materials (\%)} = \frac{W_1 \times C_1 + W_2 \times C_2}{S} \times 100$$

where

W_1 is the weight of flavoring materials to be used in powder form (g);
W_2 is the weight of flavoring materials to be used as an extract (g);
C_1 is the dried solids content of flavoring materials to be used in powder form (%);
C_2 is the dried solids content of flavoring materials to be used as an extract (%);
S is the net contents of the product (g).

4.6 Ingredients

Only the following ingredients may be used:

a) flavoring material:

powder or extracts of *katsuo-bushi* (*fushi* of bonito), *sodakatsuo-bushi* (*fushi* of frigate mackerel), *saba-bushi* (*fushi* of mackerel), *aji-bushi* (*fushi* of horse mackerel) and *iwashi-bushi* (*fushi* of sardine); extracts of bonito, frigate mackerel and mackerel; and powder or extracts of boiled and dried sardine, boiled

and dried flying fish, *kombu*, shell ligaments and dried *shiitake*;

b) sorts of sugar:

sugar, glucose, fructose, high fructose (<50 %) syrup, high fructose (≥50 %) syrup, high fructose (≥90 %) syrup, sugar added high fructose (<50 %) syrup, sugar added high fructose (≥50 %) syrup, sugar added high fructose (≥90 %) syrup and lactose;

c) salt;

d) protein hydrolysate and yeast extracts;

e) starch and dextrin.

4.7 Additives

The additives shall be as follows:

a) They shall conform to the provisions of 3.2 of CODEX STAN 192, and the conditions of use conform to the provisions of 3.3 of the document;

b) The amounts of use shall be accurately recorded and the record shall be kept;

c) Information that the additives conform to the provision of a) shall be provided to general consumers by one of the following methods; provided, however, that this does not apply to the cases where additives are added to products for business use:

1) methods of making it available for public inspection via the internet;

2) methods of displaying it on brochures, leaflets and any other publications where it is easily seen by general consumers;

3) methods of displaying it at a place where it is easily seen by general consumers in stores;

4) methods of providing it to general consumers at their request, while clearly indicating the contact address on the products.

4.8 Net contents

The net contents shall conform to the declared weight.

5 Test methods

5.1 General

Reagents and apparatus used for the testing shall be as follows:

a) **Water,** grade A2 specified in JIS K 0557, or of equivalent or higher quality.

b) **Reagents,** conforming to standards such as the special grade of Japanese Industrial Standards.

c) **Acetonitrile,** for a high performance liquid chromatograph.

d) **Kjeldahl catalysts**, mixture of potassium sulfate, 9 g, and copper (II) sulfate pentahydrate, 1 g.

e) **1 % to 4 % boric acid solution,** prepared by dissolving boric acid in water with heating to contain 10 g to 40 g of boric acid in 1 000 mL.

f) **Bromocresol green and methyl red mixture indicator,** prepared by dissolving bromocresol green and methyl red in 95 % ethanol to contain 0,15 g of bromocresol green and 0,10 g of methyl red in 200 mL.

NOTE 1 95 % ethanol shall conform to the standards of grade 1 or above, or of equivalent or higher quality.

g) **Methyl red and methylene blue mixture indicator,** prepared by dissolving methyl red and methylene blue in 95 % ethanol to contain 0,1 g of methyl red and 0,1 g of methylene blue in 200 mL.

NOTE 2 95 % ethanol shall conform to the standards of grade 1 or above, or of equivalent or higher quality.

h) **Ethylenediaminetetraacetic acid (EDTA),** of 99 % or higher purity, with a description of the nitrogen proportion.

i) **Aspartic acid,** of 99 % or higher purity, with a description of the nitrogen proportion.

j) **Volumetric glassware**, class A specified in JIS R 3505, or of equivalent or higher quality [limited to 5.3.1 and 5.4.2 a)].

k) **Filter papers,** for quantitative analysis, specified in JIS P 3801.

l) **Solid phase extraction mini-column,** with the column, made of materials which are tolerant of organic solvent, of capacity approximately 3 mL, filled with 60 mg of the copolymer of divinylbenzene and N-vinylpyrrolidone; or having the equivalent properties for separation (Pass through 2 mL of methanol and 2 mL of water in that order, respectively, and discharge the remaining water before use.).

m) **Membrane filter,** 0,45 μm or less in pore diameter, made of the materials which are tolerant of organic solvent.

n) **High performance liquid chromatograph,** with degas chamber, column oven and differential refractometer detector.

o) **Block digestor,** with an exhaust manifold, capable of boiling a digestion tube which contains 2 to 3 boiling stones and 50 mL of water, on the heating block preset at the temperature between 400 °C to 420 °C to be used in 5.4.2 a) 1.2), within 2 min 30 s.

p) **Automatic distillation apparatus,** capable of rapidly and automatically conducting steam distillation of the Kjeldahl method (including a combined apparatus of automatic distillation apparatus and automatic titrator).

q) **Apparatus for measuring total nitrogen by combustion method,** with the following characteristics:

1) equipped with a furnace capable of keeping the operating temperature at least at 870 °C or above to pyrolyze a sample in oxygen (of 99,9 % or higher purity);

2) having a structure capable of separating free nitrogen (N_2) from other combustion products to measure nitrogen (N_2) with a thermal conductivity detector;

3) having a mechanism for converting nitrogen oxide (NOx) to nitrogen (N_2);

4) having an average value of the nitrogen content within±0,15 % of the theoretical value and the relative standard deviation within 1,3 % or below in 10 consecutive measurements with nicotinic acid (of 99 % or higher purity);

5) having measures taken so that samples with a high salt concentration (about 35 %) can be measured.

5.2 Sugar content

5.2.1 General

Carry out the measurement of the sugar content with a high performance liquid chromatograph.

5.2.2 Preparation of the mixed reference solution

The preparation of the mixed reference solution shall be as follows:

a) Weigh, with accuracy, approximately 1 g of fructose, glucose and sucrose, and approximately 1,1 g of lactose monohydrate, respectively, which have been dried for 3 h at 60 °C at 2,7 kPa or less by a vacuum oven in advance; and, by obtaining a constant volume in a 100 mL volumetric flask with 50 % ethanol, prepare 10 mg/mL mixed reference solution;

b) By diluting, with accuracy, a) with 50 % ethanol, prepare 0,2 mg/mL, 1,0 mg/mL, 2,0 mg/mL, 4,0 mg/mL and 6,0 mg/mL diluted mixed reference solutions;

c) Calculate, with accuracy, each concentration of a calibration curve, using the mass which is weighed with accuracy.

5.2.3 Preparation of the sample solution

The preparation of the sample solution shall be as follows:

a) Weigh, with accuracy, approximately 5 g of the sample, obtain a constant volume in a 50 mL volumetric flask with water, and mix well. Carry out a filtration with a filter paper;

b) Let 2,5 mL of the filtrate run through a solid phase extraction mini-column, and eject the remaining filtrate. Next, let 2,5 mL of water run through the mini-column, and eject the remaining water. Receive the whole volume of solution which runs through the mini-column with a 25 mL volumetric flask, obtain a constant volume with ethanol, and mix well;

c) Carry out a filtration with a filter membrane and use the filtrate as the sample solution.

5.2.4 Condition of high performance liquid chromatograph

The condition of the high performance liquid chromatograph shall be as follows:

a) **Analytical column**, equipped with a stainless-steel tube, 4,6 mm in inner diameter and 250 mm in length, filled with polyvinyl alcohol gel or silica gel chemically bonded with polyamine; or, having the equivalent properties for separation. It shall be confirmed that, when the diluted mixed reference

solution is measured, the resolution specified in JIS K 0124 is 1,5 or more for each sugar, and, when the sample solution is measured, there are no peaks to obstruct the determination.

b) Guard column, equipped with the same column packings as the analytical column when used.

c) Column temperature, at a constant degree around 30 °C.

d) Mobile phase, 60 % to 80 % acetonitrile, the mixing ratio being constant.

e) Flow rate, at a constant speed of 0,5 mL/min to 1,5 mL/min [Make small adjustments on d) and e) so that the retention times of standard lactose is to be around 10 min to 20 min].

5.2.5 Calculation

Calculation of the sugar content shall be as follows:

a) Inject 20 μL of the diluted mixed reference solution into a high performance liquid chromatograph, measure peak areas for each sugar by the automatic integration method, using a data system, and obtain a calibration curve of concentration and peak area (Do not include the origin on the calibration curve.);

b) Inject 20 μL of the sample solution into a high performance liquid chromatograph, measure peak areas for each sugar by the automatic integration method, using a data system, and calculate the concentrations of each sugar from the calibration curve. Calculate the content of each sugar by the following formula:

$$\text{Content of each sugar (mg/g)} = \frac{A \times 25 \times 20}{W}$$

where

A is the concentration of each sugar of the sample solution, calculated from the calibration curve (mg/mL);

W is the mass of the sample (g).

c) Calculate the total sugar content by adding the contents of each sugar, and the percentage of the total sugar content to the total sample shall be the sugar content.

5.3 Salt content

5.3.1 Measurement

Measurement of the salt content shall be as follows:

a) Preparation of the sample solution

Weigh, with accuracy, approximately 5 g of the samples in a weighing dish, and obtain a constant volume in a 500 mL volumetric flask with water. Carry out a filtration with a filter paper and use the filtrate as the sample solution.

b) Titration

The titration method shall be one of the followings:

1) Automatic titration (method using a potentiometric titrator)

Pour 10 mL of the sample solution into a 100 mL beaker with a volumetric pipette; add 50 mL of dilute nitric acid (prepared by diluting 10 mL of nitric acid with water to 1 L); add 1 mL of 1 % polyoxyethylene sorbitan monolaurate with a volumetric pipette and attach to the potentiometric titrator; and while stirring the solution, titrate with 0,1 mol/L silver nitrate standard solution. Detect the end point, following the operation procedure of the titrator. As a blank test, carry out the same procedure with 10 mL of water in place of the sample solution. When the end point is not detected in the blank test, the volume required for the titration shall be 0 mL.

2) Manual titration (colorimetric observation)

Pour 10 mL of the sample solution into a 200 mL conical flask with a volumetric pipette; add 50 mL of water; add 1 mL of 2 % potassium chromate solution as an indicator; titrate with 0,05 mol/L silver nitrate standard solution, using a 25 mL brown burette. The end point shall be when the sample solution turns pale orange. As a blank test, carry out the same procedure with 10 mL of water in place of the sample solution.

5.3.2 Calculations

The salt content is obtained by the following formulae:

a) Automatic titration

$$\text{Salt content (\%)} = \frac{0{,}005\ 844 \times (T - B) \times F}{W} \times 50 \times 100$$

where

T is the volume of 0,1 mol/L silver nitrate standard solution required for the titration of the sample solution (mL);

B is the volume of 0,1 mol/L silver nitrate standard solution required for the titration of the blank test (mL);

F is the factor of 0,1 mol/L silver nitrate standard solution;

W is the mass of the sample (g);

0,005 844 is the mass of salt equivalent to 1 mL of 0,1 mol/L silver nitrate standard solution (g).

b) Manual titration

$$\text{Salt content (\%)} = \frac{0{,}002\ 922 \times (T - B) \times F}{W} \times 50 \times 100$$

where

T is the volume of 0,05 mol/L silver nitrate standard solution required for the titration

of the sample solution (mL);

B is the volume of 0,05 mol/L silver nitrate standard solution required for the titration of the blank test (mL);

F is the factor of 0,05 mol/L silver nitrate standard solution;

W is the mass of the sample (g);

0,002 922 is the mass of salt equivalent to 1 mL of 0,05 mol/L silver nitrate standard solution (g).

5.4 Total nitrogen content

5.4.1 Preparation of the sample

Those in granular form shall be used as the sample as they are, and those in powder form, etc. shall be ground and mixed by the crusher, etc., in order to prevent a change in moisture content, and homogenised to be used as the sample.

5.4.2 Measurement

Measurement shall be one of the followings:

a) The Kjeldahl method

1) Decomposition

1.1) Weigh, with accuracy, approximately 0,5 g of the sample in a weighing paper, to the nearest 0,1 mg; put it in a Kjeldahl digestion tube with a capacity between 250 mL and 300 mL, together with the weighing paper; add 10 g of Kjeldahl catalysts and 15 mL of sulfuric acid; and set it on the block digestor which has been kept warm;

1.2) Start heating at 200 °C; and, as the bubbling comes to an end after 30 min to 40 min, turn it to 400 °C to 420 °C. Check that the digestion solution has become transparent blue; and keep on heating for approximately 60 min;

1.3) After the heating is over, allow it to cool down to room temperature; add 20 mL of water and shake and mix well;

NOTE 1 When adding 20 mL or more of water after the digestion, and the amount of additional water is between 20 mL and 50 mL, adjust the amount of water which is to be added to the digestion solution of 5.4.2 a) 2) so that the total amount will be 50 mL; and, when adding 20 mL or more of water after the digestion, and the amount of additional water is over 50 mL, carry out the distillation with ammonium sulfate, etc. in advance, and make sure that the distillation will be carried out for enough time to retrieve ammonium.

1.4) As a blank test, carry out the same procedure from 1.1) to 1.3) by putting only a weighing paper in the digestion tube.

2) Distillation

Carry out the distillation following the operation procedure of the automatic distillation apparatus. When the titration is to be carried out with a burette as specified in 5.4.2 a) 3.1), place a 300 mL conical flask, to which 1 % to 4 % boric acid solution, the amount of which contains 0,3 g or more of boric acid, and a few drops of bromocresol green-methyl red mixed indicator, as a receiving liquid, are added, so that the distillate outflow port is immersed in the solution. Add 30 mL of water and 25 % to 45 % sodium hydroxide solution, the amount of which contains 24 g or more of sodium hydroxide, to the digestion solution and is alkaline, and distill it. Carry out the distillation until 150 mL or more of the distillate is obtained. Take out the outflow port out of the solution and wash the port end with a small amount of water. When the titration is to be carried out with an automatic titrator as specified in 5.4.2 a) 3.2), use, as a receiving liquid, 1 % to 4 % boric acid solution, the amount of which contains 0,3 g or more of boric acid, with or without (limited to when using an automatic titrator which does not require an indicator) an additional bromocresol green-methyl red mixed indicator or an additional methyl red-methylene blue mixed indicator. Carry out a distillation on the digestion solution obtained from the blank test in the same procedure.

NOTE 2 The sodium hydroxide solution for distillation is allowed to be below the specified amount, as far as it is possible to confirm that, at the end of the distillation, the solution will become alkaline; provided, however, that, even in this case, it is required to add, to the digestion solution obtained from the sample and the digestion solution obtained from the blank test, the sodium hydroxide solution, the amount of which is equal to those of the digestion solutions.

NOTE 3 When a mixed indicator is prepared in a different way, carry out in advance the procedures from a distillation to a titration using an ammonium sulfate, etc., and make sure that the ammonium will sufficiently be retrieved.

3) Titration

The titration method shall be one of the followings:

3.1) Manual titration

Titrate the distillate with 0,05 mol/L sulfuric acid using 25 mL or 50 mL burette. The end point shall be when the distillate turns from green to impurity-containing colorless to pale greyish red. Record the volume required for the titration to the nearest 0,01 mL. Carry out a titration on the distillate obtained from the blank test in the same procedure.

3.2) Automatic titration

Following the operation procedure of the automatic titrator (apparatus which automatically judges the end point of the titration, equipped with a burette capacity of 20 mL or more), carry out the titration on the distillate with 0,05 mol/L or 0,1 mol/L sulfuric acid. Carry out a titration on the distillate obtained from the blank test in the same procedure.

4) Calculation

Calculate the total nitrogen content by the following formula. When one drop of sulfuric acid clearly shows a color exceeding the end point in the blank test, the volume required for the titration shall be 0 mL:

$$\text{Total nitrogen content } (\%) = \frac{(T - B) \times F \times M \times A \times 2}{1\ 000 \times W} \times 100$$

where

T is the volume of the titrant required for the titration of the sample solution (mL);

B is the volume of the titrant required for the titration on the blank test (mL);

F is the factor of sulfuric acid used on titration;

M is the atomic weight of nitrogen, 14,007

A is the concentration of sulfuric acid used for the titration (mol/L);

W is the mass of the sample (g).

b) The combustion method

1) Measurement

Measurement shall be as follows:

1.1) Weigh, with accuracy, the reference standard for preparing calibration curves [use ethylenediaminetetraacetic acid (EDTA), aspartic acid, or other reference standards with the same purity (excluding nicotinic acid)] to the nearest 0,1 mg or less by following the operating procedure of the apparatus for measuring the total nitrogen content with the combustion method, carry out the measurement in an appropriate manner for the apparatus, and obtain the calibration curve;

1.2) Weigh, with accuracy, approximately 100 mg to 500 mg of the sample, to the nearest 0,1 mg, and carry out the measurement in an appropriate manner for the apparatus.

2) Calculation

Calculate the total nitrogen content (%), using the calibration curve obtained in 1.1) on the result measured in 1.2).