Tentative Translation

JAS 1703

JAPANESE AGRICULTURAL

STANDARD

Soy sauce（*Shoyu*）

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Ministry of Agriculture, Forestry and Fisheries

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Food and Agricultural Materials Inspection Center, Incorporated Administrative Agency

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Foreword

This Japanese Agricultural Standard has been revised by the Minister of Agriculture, Forestry and Fisheries through deliberations at the Council for the Japanese Agricultural Standards as the result of proposal for revision of Japanese Agricultural Standard submitted by JAPAN SOY SAUCE ASSOCIATION with the original bill being attached, based on the provision of Article 4, paragraph (1) of the Act on Japanese Agricultural Standards as applied mutatis mutandis pursuant to the provision of Article 5 of the Act. This edition replaces the previous edition of JAS for Soy sauce (*Shoyu*) (JAS 1703:2019), which has been technically revised.

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JAPANESE AGRICULTURAL STANDARD JAS
(Tentative Translation) 1703 : 2021

Soy sauce (*Shoyu*)

1 Scope

This document specifies the quality of *koikuchi shoyu*, *usukuchi shoyu*, *tamari shoyu*, *saishikomi shoyu* and *shiro shoyu* among the types of soy sauce.

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 0061, *Test methods for density and relative density of chemical products*

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

JIS K 0970, *Piston pipettes*

JIS R 3505, *Volumetric glassware*

JIS Z 8781-4, *Colorimetry — Part 4: CIE 1976 L*a*b* Colour space*

3 Terms and definitions

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

3.1
sorts of sugar
sugar, molasses and sugars

3.2
shoyu koji
mixture prepared by cultivating *koji* mold with soybeans (including defatted soybeans; the same applies hereinafter), or soybeans and grains such as wheat, rice, etc. (including those to which wheat gluten is added) which have been processed by steaming or other methods

3.3
moromi
mixture prepared by adding brine or *kiage* to *shoyu koji*, *shoyu koji* with steamed or puffed rice, or, *shoyu koji* with these rice saccharified by *koji* mold

3.4
kiage
raw liquid pressed from a fermented and aged *moromi*

3.5
hydrolyzed vegetable protein
seasoning solution prepared by processing vegetable protein of soybeans, etc. with acid

3.6
enzyme decomposed vegetable protein
seasoning solution prepared by processing vegetable protein of soybeans, etc. with proteolytic enzymes

3.7
fermented and decomposed vegetable protein
seasoning solution prepared by fermenting and decomposing wheat gluten

3.8
soy sauce
following product (including those prepared by supplementarily adding sorts of sugar, alcohol, etc.):

a) product made by the honjozo fermenting method

clear liquid seasoning obtained by fermentation and aging of *moromi* [including those made by auxiliary use of enzymes such as cellulase (for proteolytic enzymes, limited to those that are used during the heating process of *kiage* in order to prevent turbidity by the substance which mostly consists of the protein of *shiro shoyu*) during the manufacturing process];

b) product made by the mixed fermenting method

clear liquid seasoning obtained by fermentation and aging of *moromi* to which hydrolyzed vegetable protein, enzyme decomposed vegetable protein or fermented and decomposed vegetable protein has been added;

c) product made by the mixing method

clear liquid seasoning prepared by adding hydrolyzed vegetable protein, enzyme decomposed vegetable protein or fermented and decomposed vegetable protein, or a mixture of two or more of these to a), b) or *kiage*, or a mixture of two or more of these.

3.9
koikuchi shoyu
soy sauce, whose *shoyu koji* are made from soybeans and approximately the same amount of wheat or the like as soybeans, with or without grains such as rice

3.10
usukuchi shoyu
soy sauce, whose *shoyu koji* are made from soybeans and approximately the same amount of wheat or the like as soybeans with or without grains such as rice or wheat gluten; and whose *moromi* is prepared with or without steamed rice or puffed rice, or these rice saccharified by *koji* mold; and whose darkening of color and luster is suppressed during the manufacturing process

3.11
tamari shoyu
soy sauce, whose *shoyu koji* are made from soybeans or soybeans with a small amount of wheat or the like, with or without grains such as rice

3.12
saishikomi shoyu
soy sauce, whose *shoyu koji* are made from soybeans and approximately the same amount of wheat or the like as soybeans, with or without grains such as rice; and, whose *moromi* is prepared using *kiage* instead of brine

3.13
shiro shoyu
soy sauce, whose *shoyu koji* are made from wheat or the like and a small amount of soybeans, with or without wheat gluten; and whose darkening of color and luster is strongly suppressed during the manufacturing process

3.14
proportion of use of hydrolyzed vegetable protein, etc.
proportion of the total nitrogen in hydrolyzed vegetable protein, enzyme decomposed vegetable protein and fermented and decomposed vegetable protein used as raw materials to the total nitrogen in the product

4 Quality

4.1 *Koikuchi shoyu*

The quality of *koikuchi shoyu* shall conform to the quality criteria for each classification of Table 1.

Table 1 — Quality criteria for each classification of *koikuchi shoyu*

Category	Criteria		
	Special grade	**Superior grade**	**Normal grade**
Manufacturing method	The *honjozo* fermenting method	—	—
Property	Having a unique bright red-orange color with translucency, which is the characteristic of well-fermented and well-aged brewed *koikuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being excellent; and being free from objectionable taste and odor, and mold	Having a unique bright red-orange color with translucency, which is the characteristic of well-fermented and well-aged brewed *koikuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold	Having a unique bright red-orange color with translucency of *koikuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold
Degree of color	Lower than No. 18 of the standard color for soy sauce [by the color indicating method of JIS Z 8781-4, where: L^* (lightness) = 30,0, a^* = 46,1, b^* = 51,6; the same applies hereinafter] when tested by the method specified in 5.2; however, for those not heated, and sterilized by not heating but equally sterilized by another process, lower than No. 22 of the standard color for soy sauce [by the color indicating method of JIS Z 8781-4, where: L^* (lightness) = 36,7, a^* = 45,6, b^* = 62,9; the same applies hereinafter]	Same as on the left	Lower than No. 18 of the standard color for soy sauce when tested by the method specified in 5.2
Total nitrogen content	1,50 g/100 mL or more when tested by the method specified in 5.3	1,35 g/100 mL or more when tested by the method specified in 5.3	1,20 g/100 mL or more when tested by the method specified in 5.3

Table 1 — Quality criteria for each classification of *koikuchi shoyu* (*continued*)

Category	Criteria		
	Special grade	**Superior grade**	**Special grade**
Soluble solids excluding salt content	16 g/100 mL or more when tested by the method specified in 5.4	14 g/100 mL or more when tested by the method specified in 5.4	—
Ingredients	Not using ingredients other than those listed below: a) soybeans; b) wheat, barley and naked barley; c) rice; d) adlay; e) wheat gluten; f) salt; g) hydrolyzed vegetable protein, enzyme decomposed vegetable protein and fermented and decomposed vegetable protein; h) sorts of sugar; i) alcohol, *shochu* and *sake*; j) fermented rice seasoning, brewed vinegar, *mirin* and *mirin* type seasoning.		
Additives	Being as follows: a) They 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 are accurately recorded and the record shall be kept; c) Information that the additives conform to the provision of a) is 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.		
Net contents	Conform to the declared volume.		

4.2 *Usukuchi shoyu*

The quality of *usukuchi shoyu* shall conform to the quality criteria for each classification of Table 2.

Table 2 — Quality criteria for each classification of *usukuchi shoyu*

Category	Criteria		
	Special grade	**Superior grade**	**Normal grade**
Manufacturing method	Same as the criteria for the manufacturing method in Table 1	—	—
Property	Having a unique light red-orange color tinged with yellow, which is the characteristic of well-fermented and well-aged brewed *usukuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being excellent; and being free from objectionable taste and odor, and mold	Having a unique light red-orange color tinged with yellow, which is the characteristic of well-fermented and well-aged brewed *usukuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold	Having a unique light red-orange color tinged with yellow of *usukuchi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold
Degree of color	No. 22 or more of the standard color for soy sauce when tested by the method specified in 5.2	Same as on the left	No. 18 or more of the standard color for soy sauce when tested by the method specified in 5.2
Total nitrogen content	1,15 g/100 mL or more when tested by the method specified in 5.3	1,05 g/100 mL or more when tested by the method specified in 5.3	0,95 g/100 mL or more when tested by the method specified in 5.3
Soluble solids excluding salt content	14 g/100 mL or more when tested by the method specified in 5.4	12 g/100 mL ore more when tested by the method specified in 5.4	—
Ingredients	Same as the criteria for the ingredients in Table 1		
Additives	Same as the criteria for the additives in Table 1		
Net contents	Same as the criteria for the net contents in Table 1		

4.3 *Tamari shoyu*

The quality of *tamari shoyu* shall conform to the quality criteria for each classification of Table 3.

Table 3 — Quality criteria for each classification of *tamari shoyu*

Category	Criteria		
	Special grade	**Superior grade**	**Normal grade**
Manufacturing method	Same as the criteria for the manufacturing method in Table 1	—	—
Property	Having a unique reddish brown color, which is the characteristic of well-fermented and well-aged brewed *tamari shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being excellent; and being free from objectionable taste and odor, and mold	Having a unique reddish brown color, which is the characteristic of well-fermented and well-aged brewed *tamari shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold	Having a unique reddish brown color of *tamari shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold
Degree of color	Lower than No. 22 of the standard color for soy sauce when tested by the method specified in 5.2		
Total nitrogen content	1,60 g/100 mL or more when tested by the method specified in 5.3	1,40 g/100 mL or more when tested by the method specified in 5.3	1,20 g/100 mL or more when tested by the method specified in 5.3
Soluble solids excluding salt content	16 g/100 mL or more when tested by the method specified in 5.4	13 g/100 mL or more when tested by the method specified in 5.4	—
Ingredients	Same as the criteria for the ingredients in Table 1		
Additives	Same as the criteria for the additives in Table 1		
Net contents	Same as the criteria for the net contents in Table 1		

4.4 *Saishikomi shoyu*

The quality of *saishikomi shoyu* shall conform to the quality criteria for each classification of Table 4.

Table 4 — Quality criteria for each classification of *saishikomi shoyu*

Category	Criteria		
	Special grade	**Superior grade**	**Normal grade**
Manufacturing method	The *honjozo* fermenting method or the mixed fermenting method	—	—
Property	Having a unique reddish brown color, which is the characteristic of well-fermented and well-aged brewed *saishikomi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being excellent; and being free from objectionable taste and odor, and mold	Having a unique reddish brown color, which is the characteristic of well-fermented and well-aged brewed *saishikomi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold	Having a unique reddish brown color of *saishikomi shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold
Degree of color	Lower than No. 18 of the standard color for soy sauce when tested by the method specified in 5.2		
Total nitrogen content	1,65 g/100 mL or more when tested by the method specified in 5.3; however, for those made by the mixed-fermenting method, 2,00 g/100 mL or more	1,50 g/100 mL or more when tested by the method specified in 5.3	1,40 g/100 mL or more when tested by the method specified in 5.3
Proportion of use of hydrolyzed vegetable protein, etc. (limited to the products made by the mixed fermenting method)	20 % or less	—	—
Soluble solids excluding salt content	21 g/100 mL or more when tested by the method specified in 5.4	18 g/100 mL or more when tested by the method specified in 5.4	—
Ingredients	Same as the criteria for the ingredients in Table 1		
Additives	Same as the criteria for the additives in Table 1		
Net contents	Same as the criteria for the net contents in Table 1		

4.5 *Shiro shoyu*

The quality of *shiro shoyu* shall conform to the quality criteria for each classification of Table 5.

Table 5 — Quality criteria for each classification of *shiro shoyu*

Category	Criteria		
	Special grade	**Superior grade**	**Normal grade**
Manufacturing method	Same as the criteria for the manufacturing method in Table 1	—	—
Property	Having a unique light amber color, which is the characteristic of well-fermented and well-aged brewed *shiro shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being excellent; and being free from objectionable taste and odor, and mold	Having a unique light amber color, which is the characteristic of well-fermented and well-aged brewed *shiro shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold	Having a unique light amber color of *shiro shoyu*; a characteristic flavor, mellowed saltiness and *umami*; with all of them being good; and being free from objectionable taste and odor, and mold
Degree of color	No. 46 or more of the standard color for soy sauce [by the color indicating method of JIS Z 8781-4, where: L^* (lightness) = 76,7, a^* = 12,5, b^* = 81,9] when tested by the method specified in 5.2		
Total nitrogen content	0,40 g/100 mL or more and less than 0,80 g/100 mL when tested by the method specified in 5.3	0,40 g/100 mL or more and less than 0,90 g/100 mL when tested by the method specified in 5.3	Same as on the left
Soluble solids excluding salt content	16 g/100 mL or more when tested by the method specified in 5.4; this value shall not include that of added sorts of sugar	13 g/100 mL or more when tested by the method specified in 5.4	10 g/100 mL or more when tested by the method specified in 5.4
Direct reducing sugar content	12 g/100 mL or more when tested by the method specified in 5.5	9 g/100 mL or more when tested by the method specified in 5.5	6 g/100 mL or more when tested by the method specified in 5.5
Ingredients	Not using ingredients other than those listed below: a) soybeans; b) wheat, barley and naked barley; c) wheat gluten; d) salt; e) hydrolyzed vegetable protein, enzyme decomposed vegetable protein and fermented and decomposed vegetable protein; f) sorts of sugar; g) alcohol, *shochu* and *sake*; h) fermented rice seasoning, brewed vinegar, *mirin* and *mirin* type seasoning.		
Additives	Same as the criteria for the additives in Table 1		
Net contents	Same as the criteria for the net contents in Table 1		

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) **Kjeldahl catalysts,** mixture of potassium sulfate and copper (II) sulfhate pentahydrate in the ratio of 9 to 1.

d) **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

e) **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.

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

g) **DL-aspartic acid,** of 99 % or higher purity, with a description of the nitrogen proportion.

h) **Nitric acid (1+1),** prepared by adding nitric acid to an equal volume of water.

i) **Tween 20 solution,** prepared by weighing 1 g to 2 g of Tween 20, and mixing it with 100 mL of water with a measuring cylinder.

j) **Volumetric glassware,** class A specified in JIS R 3505, or of equivalent or higher quality.

k) **Variable power digestion apparatus,** capable of heating a Kjeldahl flask and boiling the Kjeldahl flask which contains 3 to 5 boiling stones and 50 mL of water within 5 min on a heat source which has been maintained at the maximum output for 10 min.

l) **Block digester,** capable of boiling 50 mL of water in a digestion tube at 420 °C within 2 min and 30 s.

m) **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).

n) **Automatic titrator,** capable of automatically judging the end point of titration, with a burette capacity of 20 mL or more.

o) **Apparatus for measuring total nitrogen by the 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) or on a dedicated apparatus for soy sauce, at least at 680 °C or above in oxygen-helium mixed gas;

   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, in 10 consecutive measurements with nicotinic acid (of 99,9 % or higher purity) or lysine hydrochloride (of 99,9 % or higher purity) and the relative standard deviation with nicotinic acid at 1,3 % or below, or with lysine hydrochloride at 0,98 % or below;

5) having measures taken so that samples with a salt concentration of approximately 20 % can be measured.

p) **Potentiometric titrator,** with a burette capacity of 20 mL or more [for the electrode, an indicator electrode (silver electrode, etc.) and a reference electrode, both suitable for chloride measurement, or a composite electrode thereof is used],

q) **Brown burette,** of capacity 25 mL or more.

5.2 Degree of color

Pour the sample into a test tube of 10 mm in diameter and compare the color with the standard colors for soy sauce.

5.3 Total nitrogen content

5.3.1 General

The total nitrogen content shall be measured by the Kjeldahl method or the combustion method.

5.3.2 The Kjeldahl method

5.3.2.1 Measurement procedure

The measurement procedure shall be as follows:

a) **Digestion of sample** The digestion of the sample shall be one of the following. The volumeter for taking a sample shall be either the air displacement type or the positive displacement type with a piston, with its systematic error being within ±1,0 % and its random error being 0,3 % or less when the selected volume is set at 1 mL and they are calculated in accordance with JIS K 0970. When such volumeter is not available, calculate the volume of the sample (mL) by measuring the density of the sample in accordance with JIS K 0061 and accurately weighing approximately 1,0 g to 1,3 g of the sample to the nearest 0,1 mg. When using an air displacement volumeter for taking the sample, apply reverse-pipetting (the method for sampling the selected volume with a pipette with the second blowout stop which dispenses the liquid in the two steps of first aspirating the sample solution fully to the second blowout stop, and then dispensing the solution to the first stop to leave the residue of the sample solution in the tip).

   1) **In the case of using a variable power digestion apparatus** Pour 1 mL of the sample into a Kjeldahl flask with a capacity between 50 mL and 300 mL, and add 5 g of Kjeldahl catalysts and approximately 8 mL of sulfuric acid. Heat the mixture gently with the variable power digestion apparatus until bubbling becomes calm, and then maximize its output. After the digestion solution becomes transparent, keep on heating for approximately 90 min more. The total heating time shall be at least 2 h. After the digestion is over, allow it to cool down to room temperature, add 20 mL to 50 mL of water according to the most manageable volume of the following distillation apparatus, and dissolve the sample with low heating if needed. For the blank test, carry out the same procedure without a sample.

   2) **In the case of using a block digester** Pour 1 mL of the sample into a digestion tube with a capacity between 250 mL and 300 mL, and add 5 g of Kjeldahl catalysts and approximately 8 mL of sulfuric acid. Heat the mixture in a block digester preset at 200°C until bubbling becomes calm, and then reset the block digester at 420 °C. After the digestion solution becomes transparent, keep on heating for approximately 90 min more. After the digestion is over, allow it to cool down to room temperature, add 20 mL to 50 mL of water according to the most manageable volume of the following distillation apparatus, and dissolve the sample with low heating if needed. For the blank test, carry out the same procedure without the sample. If the block digester is equipped with an exhaust manifold, etc. to emit a generated sulfuric acid mist, and the safety of the analysing condition is ensured, the digestion may be started from 420° C by adding 1 mL of 30 % hydrogen peroxide after pouring sulfuric acid.

If there is a risk of the digestion solution leaking out by bubbling, etc., stop the heating, cool it until bubbling becomes calm, and then restart the heating.

b) **Distillation** The distillation method shall be one of the following:

1) **In the case of using a Shioiri–Okuda type distillation apparatus** Pour 25 mL to 30 mL of boric acid solution into a receiver for distillate (hereinafter referred to as "receiver"), add a few drops of bromocresol green and methyl red mixture indicator, and place it so that the distillate outflow port is immersed in the solution of the receiver. Connect the Kjeldahl flask containing the digestion solution to the distillation apparatus, add 25 % to 45 % sodium hydroxide solution to the digestion solution so that it contains 16 g or more of sodium hydroxide and is alkaline, and distil it until 100 mL or more of the distillate is obtained. Take the outflow port out of the solution and wash the port end with a small amount of water.

2) **In the case of using a Parnas–Wagner type distillation apparatus and distilling all of the digestion solution** Pour 25 mL to 30 mL of boric acid solution into the receiver, add a few drops of bromocresol green and methyl red mixture indicator, and place it so that the distillate outflow port is immersed in the solution of the receiver. Pour the digestion solution into a distillation tube and wash the inner side of the Kjeldahl flask or the digestion tube with water three times. The volume of the water is 30 mL or more according to the capacity of the distillation tube. Add 25% to 45% sodium hydroxide solution to the digestion solution so that it contains 16 g or more of sodium hydroxide and is alkaline, and distil it until 100 mL or more of the distillate is obtained. Take the outflow port out of the solution and wash the port end with a small amount of water.

3) **In the case of using a Parnas–Wagner type distillation apparatus and distilling part of the digestion solution** Pour the digestion solution into a 100 mL volumetric flask and wash the inner side of the Kjeldahl flask or the digestion tube with water and add water to the graduation line. Use this as the sample solution. Pour 25 mL to 30 mL of boric acid solution into the receiver, add a few drops of bromocresol green and methyl red mixture indicator and place it so that the distillate outflow port is immersed in the solution of the receiver. Pour 25 mL of the sample solution by using a volumetric pipette into the distillation tube, add 25 % to 45 % sodium hydroxide solution to the sample solution so that it contains 4 g or more of sodium hydroxide and is alkaline, and distil it until 100 mL or more of the distillate is obtained. Take the outflow port out of the solution and wash the port end with a small amount of water.

4) **In the case of using an automatic distillation apparatus** Pour 25 mL to 30 mL of boric acid solution into the receiver, add a few drops of bromocresol green and methyl red mixture indicator and place it so that the distillate outflow port is immersed in the solution of the receiver. Add 30 mL of water and 25 % to 45 % sodium hydroxide solution to the digestion solution so that it contains 16 g or more of sodium hydroxide and is alkaline, and distil it until 100 mL or more of distillate is obtained by following the operational procedure of the automatic distillation apparatus. Take the outflow port out of the solution and wash the port end with a small amount of water. In the case of using a combined apparatus of automatic distillation apparatus and automatic titrator, carry out the distillation and titration by a procedure suitable for the apparatus.

c) **Titration** The titration method shall be one of the following:

1) **Manual titration (a method in which the end point of titration is visually judged by the discoloration of the indicator)** For the distillate obtained by distilling part of the digestion solution using a Parnas–Wagner type distillation apparatus, titrate it with 0,025 mol/L sulfuric acid using a 25 mL burette. For other distillates obtained by distilling all of the digestion solution, titrate it with 0,05 mol/L sulfuric acid using a 25 mL burette. The moment when the color of the liquid turns to pale grayish red through green and impurity-

containing colorless is the end point of titration. Record the titration value to the nearest 0,01 mL. For the distillate obtained from the blank test, titrate it by the same procedure.

2) **Automatic titration (a method in which the end point of titration is automatically judged)** Titrate the distillate following the operation procedure of the automatic titrator. For the distillate obtained from the blank test, titrate it by the same procedure.

5.3.2.2 Calculations

The total nitrogen content is obtained by the following formulae. When one drop of sulfuric acid clearly shows a color exceeding the end point in the blank test, the titration value shall be 0 mL.

a) **In the case of distilling and titrating all of the digestion solution**

$$N = \frac{(T - B) \times F \times M \times A \times 2}{1\,000 \times V} \times 100$$

b) **In the case of distilling and titrating part of the digestion solution**

$$N = \frac{(T - B) \times F \times M \times A \times 2}{1\,000 \times V} \times \frac{100}{25} \times 100$$

where

N is the total nitrogen content (g/100 mL);

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

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

F is the factor of titrant;

M is the atomic weight of nitrogen, 14,007;

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

V is the volume of the sample (mL).

5.3.3 The combustion method

5.3.3.1 Measurement procedure

The measurement procedure shall be as follows:

a) Following the operation procedure of each apparatus for measuring total nitrogen by the combustion method, accurately weigh the reference standard for preparing calibration curves {using ethylenediaminetetraacetic acid (EDTA), DL-aspartic acid or other reference standards with the same purity [excluding the standard used in 5.1 o) 4)]} to the nearest 0,1 mg or less. Then measure it by a procedure suitable for the apparatus, and prepare a calibration curve.

b) Following the operation procedure of each apparatus for measuring total nitrogen by the combustion method, accurately weigh the sample to the nearest 0,1 mg for 100 mg or more of the sample, or to the nearest 0,01 mg for less than 100 mg but 40 mg or more of the sample. Then measure it by a procedure suitable for each apparatus. The method for taking the sample may be replaced with the method using a volumeter which can accurately measure the selected volume (either the air displacement type volumeter with its air layer capacity being 25 % or less of the selected volume, or the positive displacement type volumeter with a piston, with its systematic error being within ± 1,0 % and its random error being 0,3 % or less when the selected volume is set and they are calculated in accordance with JIS K 0970).

c) Measure the density of the sample in accordance with JIS K 0061.

5.3.3.2 Calculations

The total nitrogen content is obtained by the following formula:

$$N = \frac{n}{m} \times 100 \times \rho$$

where

N is the total nitrogen content (g/100 mL);

n is the mass of nitrogen obtained from the calibration curve (mg);

m is the mass of the sample (mg);

ρ is the density of the sample (g/mL).

When the sample is taken by the volumeter in 5.3.3.1 b), the total nitrogen content is obtained by dividing the mass of nitrogen obtained from the calibration curve by the selected volume.

5.4 Soluble solids excluding salt content

5.4.1 Measurement of the soluble solids content

The soluble solids content shall be the reading with a sugar refractometer, on condition that both the sample and the refractometer are kept at 20 °C, expressed as a percentage.

5.4.2 Measurement of the salt content

5.4.2.1 General

The salt content shall be measured by the potentiometric titration method or the Mohr method.

5.4.2.2 Preparation of the sample solution

Pour 5 mL of sample into a 250 mL volumetric flask with a volumetric pipette and add water to the graduation line. Use this as the sample solution. The sample solution may be replaced with the sample obtained by using a device which is capable of accurately measuring a constant volume and then diluted with water.

5.4.2.3 Titration

The titration method shall be one of the following:

a) **Potentiometric titration method** Pour 10 mL of the sample solution into a beaker with a capacity between 100 mL and 200 mL with a volumetric pipette, add water to immerse the electrode in the sample solution, add 1 mL of nitric acid (1 + 1) and 1 mL of Tween 20 solution, and mount the beaker to a potentiometric titrator. While stirring the solution, titrate it with 0.05 mol/L or 0.1 mol/L of silver nitrate solution, and determine the end point in accordance with the operation procedure of the titrator. For the blank test, titrate water as a substitute for the sample solution by the same procedure. When no end point is detected or the volume of the silver nitrate solution required for titration is less than 0.01 mL in this blank test, the titration value shall be 0 mL. Provided that the end point of the potentiometric titrator is able to be judged accurately, the concentration of the silver nitrate solution may be altered. As a substitute for Tween 20 solution, a solution containing an anionic surfactant suitable for the potentiometric titrator may be used.

b) **Mohr method** Pour 5 mL of the sample solution into a porcelain evaporating dish or an Erlenmeyer flask with a volumetric pipette, add 1 mL of 2 % potassium chromate solution as an indicator, and titrate it with 0,02 mol/L of silver nitrate solution with a Brown burette. The moment when the color of the liquid turns to pale orange is the end point of titration. For the blank test, titrate 5 mL of water as a substitute for the sample solution by the same procedure. When one drop of the silver nitrate clearly shows a color exceeding the end point in this blank test, the titration value shall be 0 mL.

5.4.2.4 Calculations

The salt content is obtained by the following formulae. When the sample solution is obtained by using a device which is capable of accurately measuring a constant volume and then diluted with water, as described in 5.4.2.2, eliminate "× (250/10)" from formula a) and "× (250/5)" from formula b):

a) **Potentiometric titration method**

$$D = \frac{T-B}{1\,000} \times A \times F \times M \times \frac{250}{10} \times \frac{1}{V} \times 100$$

b) **Mohr method**

$$D = \frac{T-B}{1\,000} \times A \times F \times M \times \frac{250}{5} \times \frac{1}{V} \times 100 \times C$$

where

D is the salt content (g/100 mL);

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

B is the volume of silver nitrate solution required for the titration on the blank test (mL);

A is the concentration of silver nitrate solution used for the titration (mol/L);

F is the factor of silver nitrate solution;

M is 58,44 (the formula weight of sodium chloride);

V is the volume of the sample (mL);

C is the correction factor (*shiro shoyu*: 1,00; *usukuchi shoyu*: 0,99; *koikuchi shoyu*: 0,98; *tamari shoyu* and *saishikomi shoyu*: 0,97).

5.4.3 Calculation of soluble solids excluding salt content

The soluble solids excluding salt content is the value obtained by subtracting the salt content (see 5.4.2) from the soluble solids content (see 5.4.1).

5.5 Direct reducing sugar

The measurement of direct reducing sugar shall be as follows:

a) Dilute 10 mL of the sample 20 to 25 times, take 5 mL to 20 mL of the diluted solution, add 20 mL of Fehling's solution and water so that the total volume is 50 mL, boil the solution accurately for 2 min, and then cool it rapidly;

b) Add 10 mL of 25 % sulfuric acid and 3 g of potassium iodide to the solution, then titrate it with 0,1 mol/L sodium thiosulfate solution using 1 % starch solution as an indicator. Carry out the blank test without adding samples, and the difference between the values of both titrations is the direct reducing sugar.