Tentative Translation

JAS 0009

JAPANESE AGRICULTURAL STANDARD

Determination of the lycopene in raw tomato

— **Spectrophotometric method**

Date of Establishment: 2019-1-31
Date of Revision: 2024-7-31

Ministry of Agriculture, Forestry and Fisheries

Precautions for using English version of JAS

This English translation has been made by the drafting party etc., based on the original text (Japanese version), and has been posted on the website of the Food and Agricultural Materials Inspection Center (FAMIC), Incorporated Administrative Agency, with permission of the publisher of the original text (Ministry of Agriculture, Forestry and Fisheries).

The translation is made in consideration of technical contents, but it is aimed to provide information when using JAS original text, and is not recognized as having the same effects as the original text.

If there is any doubt in the translation, please follow the original.

FAMIC is not responsible for inconvenience by using only the translation.

Food and Agricultural Materials Inspection Center, Incorporated Administrative Agency

Contents

Page

1 Scope 1

2 Normative references 1

3 Terms and definitions 1

4 Principle 1

5 Reagents 1

6 Apparatus 2

7 Preparation of test samples 2

8 Procedure 2

8.1 General 2

8.2 Extraction 3

8.3 Dilution 4

8.4 Determination 4

9 Calculation 4

9.1 Quantitation 4

9.2 Expression of results 5

10 Precision 5

10.1 Interlaboratory test 5

10.2 Repeatability 5

10.3 Reproducibility 5

11 Quality control 5

12 Test report 5

Annex A (informative) Results of interlaboratory test 6

Bibliography 7

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 a result of proposal for the revision of Japanese Agricultural Standard submitted by Food and Agricultural Materials Inspection Center, Incorporated Administrative Agency with the original bill being attached, based on the provisions of Article 4, paragraph (1) of the Act on Japanese Agricultural Standards as applied mutatis mutandis pursuant to Article 5 of the same Act. This edition replaces the previous edition (JAS 0009:2019), which has been technically revised.

Attention is drawn to the possibility that some parts of this document may conflict with patent rights, published patent applications or utility model rights. The Minister of Agriculture, Forestry and Fisheries and the Council for the Japanese Agricultural Standards are not responsible for identifying any such patent rights, published patent applications or utility model rights.

JAPANESE AGRICULTURAL STANDARD JAS
(Tentative Translation) 0009 : 2024

Determination of the lycopene in raw tomato

— Spectrophotometric method

WARNING — The user of this document should be familiar with normal laboratory practice. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This document specifies a spectrophotometric method for the determination of lycopene in the ripe red tomatoes (*Solanum lycopersicum*) (fresh fruits).

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.

ISO 648, *Laboratory glassware — Single-volume pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

JIS K 0115, *General rules for molecular absorptiometric analysis*

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

JIS K 8034, *Acetone (Reagent)*

JIS K 8848, *Hexane (Reagent)*

JIS K 8891, *Methanol (Reagent)*

3 Terms and definitions

No terms and definitions are listed in this document.

4 Principle

Lycopene is extracted with hexane/acetone mixture from the test sample, after washing with methanol to remove β-carotene. The sample extract is diluted to obtain the measurement solution. The lycopene in the measurement solution is determined by a spectrophotometer.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

WARNING — It is the responsibility of users of this document to comply with legal regulations

regarding the use of reagents.

5.1 Water, conforming to grade A2, A3, or A4 of JIS K 0557.

5.2 Filter aid, flux-calcined diatomaceous earth. Without elution of obstacle to analysis.

5.3 Methanol, of minimum mass fraction, 99,8 %, according to JIS K 8891.

5.4 Hexane, of minimum mass fraction, 96,0 %, according to JIS K 8848.

5.5 Acetone, of minimum mass fraction, 99,5 %, according to JIS K 8034.

5.6 Hexane/acetone mixture, mix 9 parts per volume of hexane with 1 part per volume of acetone.

6 Apparatus

The usual laboratory apparatus and the following shall be used.

6.1 Electronic analytical balances, capable of weighing to an accuracy of ±10 mg.

6.2 Beakers, made of glass, of approximately 20 ml capacity.

6.3 Funnels, Buchner funnels with a fritted glass disc of approximately about 30 mm in diameter and a pore size of approximately 16 μm to 40 μm.

6.4 Glass rods, of adequate length and diameter to stir and compact the test sample and filter aid in the funnels.

6.5 Vacuum filtering device, depressurizing system (for example, aspirator) with a filtering bell jar of adequate size to equip funnels and one-mark volumetric flasks.

6.6 One-mark volumetric flasks, amber, to cover the volume range for extraction and dilution, of ISO 1042, class A.

6.7 Single-volume pipettes, to cover the volume range for dilution, of ISO 648, class A.

6.8 Spectrophotometer, capable of measuring absorbance at a wavelength of 472 nm and holding absorption cells, of JIS K 0115.

6.9 Absorption cells, made of quartz glass or glass, and should have stoppers. When using multiple cells, optical characteristics shall be equivalent.

6.10 Membrane filters, made of polytetrafluoroethylene (PTFE), suitable for organic solvents, with a pore size of 0,45 μm or less. The filter and the housing shall be unitary, and the housing material shall be resistant to organic solvents.

7 Preparation of test samples

After removing the calyx of the sample, crush it using a homogenizer or the like to obtain the test sample. Immediately perform the procedure in 8.2, or freeze the test sample to store. When storing the test sample frozen, transfer all of them, or a portion of them stirred until homogeneous, into a sealable glass container soon after crushed. Return it to room temperature and mix well before use.

NOTE It has been confirmed that the test samples remain stable for at least 4 weeks when stored in a sealed amber glass container, frozen at -30 °C to -20 °C.

8 Procedure

8.1 General

In order to avoid decomposition of lycopene by light, the procedure should be carried out in a place that is not exposed to direct sunlight or strong artificial light.

8.2 Extraction

8.2.1 General

At vacuum filtering process, the filter aid layer can be compacted insufficiently with a glass rod due to clogging of glass filter of funnels or weak vacuuming power of the vacuum filtering device. In this case, the sample extract (see 8.2.4.6) can separate into two phases due to water contamination and accordingly, fixing volume accurately becomes difficult. Therefore, use the apparatus capable of performing the operation in 8.2.2.1. Perform the procedures in 8.2.2.3 to 8.2.4.6 quickly without interruption.

8.2.2 Preparation of extraction

8.2.2.1 Attach funnel to the vacuum filtering device. Wet the glass filter of the funnel with a small amount of water. Add filter aid to the funnel and add water up to approximately 80 % of the funnel volume. Stir well with a glass rod and depressurize. Compact the filter aid with a glass rod and form a filter aid layer of 5 mm to 8 mm. Return the internal pressure of the vacuum filtering device to atmospheric value.

8.2.2.2 Mix well the test sample (see Clause 7), weigh, to the nearest 10 mg, approximately 5 g into a beaker. If it takes time to start the procedure in 8.2.2.3, such as when preparing multiple test samples, the test sample should be shielded from light (for example, by covering up the beaker with aluminum foil).

8.2.2.3 Add filter aid, approximately one-half to the same of the amount used in 8.2.2.1, to the test sample in the beaker, stir the mixture well with a glass rod, and use as the filter aid mixed sample.

8.2.2.4 Add the filter aid mixed sample to the funnel (8.2.2.1). Transfer the entire residue in the beaker to the funnel using a small amount of water.

8.2.2.5 Add water to the funnel (8.2.2.4) up to approximately 80 % of the funnel volume, and then stir well only the filter aid mixed sample with a glass rod. Herein, take care not to break the filter aid layer that formed in 8.2.2.1.

8.2.2.6 Perform vacuum filtering, and discard the filtrate. Compact the filter aid mixed sample with a glass rod to form a layer. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

NOTE Longer vacuum filtering can accelerate lycopene decomposition by oxygen and reduce its content.

8.2.3 Removal of β-carotene

8.2.3.1 Add approximately 10 ml of methanol to the funnel while allowing the methanol to wash the funnel's inner surface. Stir the upper layer, i.e., the filter aid mixed sample layer, with a glass rod. Herein, take care not to break the lower layer formed in 8.2.2.1, i.e., the filter aid layer.

8.2.3.2 After leaving to stand for 1 minute, perform vacuum filtering, and discard the filtrate. Compact the filter aid mixed sample with a glass rod and re-form a filter aid mixed sample layer. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

8.2.3.3 Repeat the procedures in 8.2.3.1 and 8.2.3.2 two more times.

NOTE It has been confirmed that almost all the β-carotene in the test sample is removed by the procedures in 8.2.3.1 to 8.2.3.3 [4].

8.2.4 Extracting lycopene

8.2.4.1 Place a 50 ml one-mark volumetric flask in the vacuum filtering device.

8.2.4.2 Add approximately 10 ml of hexane/acetone mixture to the funnel while allowing the mixture to wash the funnel's inner surface. Stir both the upper layer, i.e., the filter aid mixed sample layer, and the lower layer, i.e., the filter aid layer with a glass rod.

8.2.4.3 Perform vacuum filtering, and collect the filtrate into the 50 ml one-mark volumetric flask. Compact the mixture of the test sample and the filter aid stirred in 8.2.4.2 with a glass rod. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

8.2.4.4 Repeat the procedures in 8.2.4.2 and 8.2.4.3 three more times.

8.2.4.5 Add approximately 5 ml of hexane/acetone mixture to the funnel while allowing the mixture to wash the funnel's inner surface, and perform vacuum filtering immediately. After filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

8.2.4.6 Remove the 50 ml one-mark volumetric flask from the vacuum filtering device. After it returns to room temperature, fill up to the mark with hexane/acetone mixture, shake to mix, and use as the sample extract. Perform absorbance measurement on the same day or transfer them into a sealable glass container and store at -20 °C or below. When using the stored sample extract, return it to room temperature and mix well before use.

NOTE It has been confirmed that the sample extracts remain stable for at least five days when stored in a sealed amber glass container, at -30 °C to -20 °C.

8.3 Dilution

Dilute the sample extract (see 8.2.4.6) 5-fold with hexane/acetone mixture using the single-volume pipette and the one-mark volumetric flask. Filter it through a membrane filter and use as the measurement solution. The measurement solution should be shielded from light until the procedure in 8.4 is performed.

8.4 Determination

8.4.1 General

If the absorbance of the measurement solution is not within the range of 0,2 to 1, dilute and measure again with varied dilution ratio.

8.4.2 Set up of spectrophotometer

Set up and operate the spectrophotometer in accordance with the manufacturer's instructions. Set the wavelength to 472 nm.

8.4.3 Absorbance measurement

8.4.3.1 After filling the absorption cell with hexane/acetone mixture as reference, place it in the cell holder of the spectrophotometer, and adjust absorbance to zero.

8.4.3.2 Fill the absorption cell with the measurement solution (see 8.3) after prewashing the cell two times with this solution.

8.4.3.3 Place the absorption cell in the cell holder, and measure absorbance of the measurement solution.

9 Calculation

9.1 Quantitation

The lycopene content in the test sample, w, is given by the formula:

$$w = \frac{A \times V \times d \times 10^4}{E \times l \times m}$$

where

w is the lycopene content in the test sample (mg/kg);

A is the absorbance of the measurement solution determined at 472 nm (hexane/acetone mixture);

V is the constant volume (ml) at extraction (see 8.2.4);

d is the dilution ratio at dilution(see 8.3);

E is the absorption coefficient of lycopene in concentration 1% and optical path length 1 cm, 3 450[7];

l is the optical path length(cm) of the absorption cell;

m is the mass(g) of the test sample.

NOTE In the interlaboratory test described in Annex A, V was 50, and d was 5 or 10 depending on the absorbance of measurement solution.

9.2 Expression of results

Express the results to two significant figures.

10 Precision

10.1 Interlaboratory test

An interlaboratory test was carried out to determine the precision of the test method, and the results are summarized in Annex A. The values derived from this interlaboratory test can be inapplicable to the content ranges other than the given one (39 mg/kg to $1{,}7\times10^2$ mg/kg) nor the matrices other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, is expected in not more than 5 % of cases to be greater than the repeatability limit (r) values [2] given in Table A.1 as long as the specified operation is correctly done [1].

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, is expected in not more than 5 % of cases to be greater than the reproducibility limit (R) values [2] given in Table A.1 as long as the specified operation is correctly done [1].

11 Quality control

The laboratory shall have internal quality control procedures for tests.

12 Test report

The test report shall include at least the following information:

a) the title or the reference number of this document;

b) every detail to identify the test sample;

c) the date of the test;

d) the results of the test.

Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was carried out in accordance with IUPAC protocol [3] in 2018 in Japan, and gave the statistical results given in Table A.1 [4]. Commercially available or other provided tomatoes, which the calyx were removed, added the pyrogallol [5] 3 % of sample mass and crushed.

After the homogeneity [6] was confirmed, each of the crushed material was used as a test sample. The experimental protocol and test samples were supplied to the participating laboratories by the Food and Agricultural Materials Inspection Center (FAMIC), the organizer of this interlaboratory test. Each laboratory tested a total of 12 test samples (6 pairs of blind duplicates) according to the experimental protocol.

NOTE Since lycopene can be decomposed by light, oxygen, enzymes contained in samples, antioxidants were added to stabilize the lycopene concentration of the test samples during the experimental term.

Table A.1 — Precision data

Sample identification	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Number of participating laboratories	10	10	10	10	10	10
Number of accepted test results	9	9	9	10	10	10
Mean lycopene content, mg/kg (mass fraction)	38,57	45,6	61,9	97,2	118,7	168,8
Repeatability standard deviation s_r mg/kg	0,48	0,67	1,2	2,7	2,2	5,1
Repeatability relative standard deviation, RSD_r, %	1,2	1,5	2,0	2,7	1,9	3,0
Repeatability limit r ($r = 2{,}8\ s_r$) mg/kg	1,3	1,9	3,5	7,5	6,2	14
Reproducibility standard deviation s_R mg/kg	0,94	1,9	2,5	3,8	4,5	5,8
Reproducibility relative standard deviation, RSD_R, %	2,4	4,2	4,1	3,9	3,8	3,4
Reproducibility limit R ($R = 2{,}8\ s_R$) mg/kg	2,6	5,4	7,1	11	13	16

Bibliography

[1] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

NOTE Section 7.1.5 of the referenced document was referred to for the expression of the repeatability limit and the reproducibility limit.

[2] ISO 5725-6:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values*

NOTE Section 4 "Determination of limits" of the referenced document was referred to for the calculation of the repeatability limit and the reproducibility limit.

[3] Horwitz, W., Protocol for the design, conduct and interpretation of method-performance studies, *Pure & Appl. Chem.*, 1995, **67**(2), pp. 331-343.

[4] Kakubari, S., et al., Determination of Lycopene Concentration in Fresh Tomatoes by Spectrophotometry: A Collaborative Study, *J. AOAC Int.*, 2020, **103**(6), pp. 1619-1624.

[5] JIS K 8780, *Pyrogallol (Reagent)*

[6] Thompson, M., et al., The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure & Appl. Chem.*, 2006, **78**(1), pp. 145-196.

NOTE Section 3.11 "Testing for sufficient homogeneity and stability" of the referenced document was referred to for the method to confirm the homogeneity.

[7] Britton, G., Liaaen-Jensen, S., Pfander, H. ed., *Carotenoids handbook*, Birkhauser Verlag, Basel/Boston/Berlin, 2004

NOTE "MAIN LIST 31(Lycopene) Spectroscopic data" of the referenced document was referred to for the absorption coefficient of lycopene.