Tentative Translation

JAS 0009

JAPANESE AGRICULTURAL STANDARD

Determination of the lycopene in raw tomato — Spectrophotometric method

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Ministry of Agriculture, Forestry and Fisheries

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Incorporated Administrative Agency

Food and Agricultural Materials Inspection Center

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Warning — The user of this Standard should be familiar with normal laboratory practice. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard 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*) (raw).

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. For undated references, 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

JIS K 8848 Hexane

JIS K 8891 Methanol

3 Principle

Lycopene is extracted with hexane/acetone mixture from a test portion of pureed tomatoes, after washing with methanol to remove β-carotene. The concentration of lycopene in the extract is determined by a spectrophotometer.

4 Reagents

Use only reagents recognized analytical grade, unless otherwise specified.

Warning — It is the responsibility of users of this standard to comply with legal regulations regarding the use of reagents.

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

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

4.3 Methanol, of minimum mass fraction, $\varphi(CH_3OH) \geq 99{,}8$ %, according to **JIS K 8891**.

4.4 Hexane, of minimum mass fraction, $\varphi(CH_3(CH_2)_4CH_3) \geq 96{,}0$ %, according to **JIS K 8848**.

4.5 Acetone, of minimum mass fraction, $\varphi(CH_3COCH_3) \geq 99,5$ %, according to **JIS K 8034**.

4.6 Hexane/acetone mixture,

Mix 9 parts per volume of hexane (**4.4**) with 1 parts per volume of acetone (**4.5**).

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Analytical balances, capable of weighing to an accuracy of ±0,1 mg.

5.2 Beakers, glass, of approximately 20 mL capacity.

5.3 Filter Funnels, Buchner filter funnels with fritted glass disk about 30 mm in diameter and 16 μm－40 μm pore size.

5.4 Glass rods, adequate length and diameter to mix the test sample and filter aid (**4.2**) in the filter funnels (**5.3**).

5.5 Vacuum filtering device, depressurizing system (for example, aspirator) with a filtering bell gar of adequate size to equip filter funnels (**5.3**) and one-mark volumetric flasks (**5.6**).

5.6 One-mark volumetric flasks, amber, to cover the volume range for sample extraction and dilution, of **ISO1042**, class A.

5.7 Single volume pipettes, to cover the volume range for sample dilution, of **ISO648**, class A.

5.8 Spectrophotometer, capable measuring wavelength 472 nm, holding absorption cells (**5.9**).

5.9 Absorption cells, quartz glass or glass, and they should have stoppers. When more than one cell is used, use the ones that guaranteed to have same optical characteristic.

5.10 Membrane filters, for organic solutions, made of polytetrafluoroethylene (PTFE), with a pore size of less than 0,45 μm. The filter and the housing are unitary, and the material of the housing is resistant to organic solvents.

6 Preparation of test samples

After removing the calyx of the sample, it is pureed using a homogenizer or the like. This is used as a test sample. Proceed immediately in accordance with **7.2**, or store frozen the test samples. If test samples are stored frozen, transfer all of them, or a portion of them stirred until homogeneous, into the glass sealed containers soon after pureed. Remove the test samples from the freezer before use, allow them to room temperature and mix well.

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

7 Procedure

7.1 General

In order to avoid decomposition of lycopene by light, each process of lycopene determination should be carried out under weak light if possible and not be exposed to light for a long time.

7.2 Extraction

7.2.1 General

At vacuum filtering process, if the filter aid layer is too loose to push with a glass rod, this may due to closing of glass filter of filter funnels (**5.3**) or weak vacuuming power of the vacuum filtering device (**5.5**). At this time, water may keep remaining in the filter aid layer until the step **7.2.2.11** and mix into the sample extract (**7.2.2.15**). As a result,

sample extract separate into two phases and accordingly, fixing volume accurately become difficult at the step **7.2.2.15**. Use the apparatus that can conduct the process of **7.2**. On the other hand, stop vacuuming after pushing the filter aid with glass rod. Because lycopene content may become low due to decomposition on contact with air.

7.2.2 Extraction procedure

7.2.2.1 Attach a filter funnel (**5.3**) to the vacuum filtering device (**5.5**) and wet glass filter of the funnel with a small amount of water. Add the filter aid (**4.2**) to the funnel and add water of 80 % of the funnel volume. Stir well with a glass rod and depressurize. Push 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.

7.2.2.2 Mix well the test sample (**6**), weigh to the nearest 10 mg, approximately 5 g, and place in a beaker (**5.2**).

7.2.2.3 Add filter aid approximately one-half of the amount used in the process of **7.2.2.1**, to the test sample in the beaker (**7.2.2.2**) and stir the mixture well with a glass rod.

7.2.2.4 Add the mixture of the test sample and filter aid to the funnel (**7.2.2.1**). Transfer the residue in the beaker to the funnel using a small amount of water.

7.2.2.5 Add water to the funnel (**7.2.2.4**) approximately 80 % of the funnel volume is filled, and then stir well the mixture of the test sample and filter aid. Herein, take care not to break the filter aid layer that formed in the process of **7.2.2.1**.

7.2.2.6 Perform vacuum filtering, and discard the filtered liquid. Push the mixture of the test sample and filter aid with a glass rod to form a layer. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

7.2.2.7 Add approximately 10 mL of methanol to the funnel, allowing the methanol to wash the funnel's inner surface. Stir the top layer, i.e., the mixture of test sample and filter aid, with a glass rod. Herein, take care not to break the bottom filter aid layer, formed in the process of **7.2.2.1**.

7.2.2.8 After leaving to stand for 1 minute, perform vacuum filtering, and discard the filtered liquid. Push the top layer with a glass rod. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

7.2.2.9 Repeat steps **7.2.2.7** to **7.2.2.8** two times.

7.2.2.10 Place a 50 mL volumetric flask (**5.6**) in the vacuum filtering device.

7.2.2.11 Add approximately 10 mL of hexane/acetone mixture (**4.6**) to the funnel while allowing the mixture to wash the funnel's inner surface. Stir both the top and bottom layers with a glass rod.

7.2.2.12 Perform vacuum filtering, and collect the filtered liquid into the 50 mL volumetric flask. Push the mixture of the test sample and filter aid with a glass rod. Immediately after filtration, return the internal pressure of the vacuum filtering device to atmospheric value.

7.2.2.13 Repeat steps **7.2.2.11** to **7.2.2.12** three times.

7.2.2.14 Add about 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..

7.2.2.15 Remove the 50 mL volumetric flask from the vacuum filtering device. After it returns to room temperature, add to the mark with hexane/acetone mixture and mix. This is used as the sample extract. Perform absorbance measurement (**7.4**) on the day of preparation or transfer them into the glass sealed containers and store at -20 °C or

lower. Before use sample extract stored at -20 °C or lower, return to normal temperature and mix well.

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

7.3 Dilution

Dilute the sample extract (**7.2.2.15**) 5-fold with hexane/acetone mixture (**4.6**) using the single-volume pipette (**5.7**) and the one-mark volumetric flask (**5.6**). Filter it through a membrane filter (**5.10**).This is used as measurement solution.

7.4 Determination

7.4.1 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.

7.4.2 Set up of spectrophotometer

Set up and operate the spectrophotometer (**5.8**) in accordance with the manual. Set the wavelength to 472 nm.

7.4.3 Absorbance measurement

7.4.3.1 After fill the absorption cell (**5.9**) with hexane/acetone mixture (**4.6**) as reference, place it in the cell holder of the spectrophotometer (**5.8**), and adjust absorbance to zero.

7.4.3.2 Fill the absorption cell with measurement solution, after prewashing the cell two times.

7.4.3.3 Place the absorption cell (**7.4.3.2**) in the cell holder, and measure absorbance of sample extract solution.

8 Calculation

8.1 Quantitation

The lycopene content in sample, w (mg/kg), is given by the formula:

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

where

A is the absorbance of sample extract solution at 472 nm (hexane/acetone mixture (**4.6**), 1 cm cell);

V is the constant volume (mL) at extraction (**7.2**);

d is the dilution ratio at dilution(**7.3**);

E is the absorption coefficient of 1 % lycopene concentration and optical path length 1 cm, 3 450[5];

l is the optical path length(cm) of the absorption cell(**5.9**);

m is the mass(g) of the sample test portion(**7.2.2.2**).

NOTE In the interlaboratory tests described in **Annex A**, V is 50 and d is 5 to 10, depending on concentration of lycopene.

8.2 Expression of results

Express the results to two significant figures.

9 Precision

9.1 Interlaboratory test

Details of the interlaboratory test to determine the precision of the method are summarized in **Annex A**. The values derived from this interlaboratory test might not be applicable to concentration ranges (39 mg/kg to 1.7×10^2 mg/kg)

and matrices other than those given.

9.2 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, may be expected in not more than 5 % of cases be greater than the repeatability limit (r) values[2] shown in **Table A.1** on average as long as the specified operation is definitely done[1].

9.3 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, may be expected in not more than 5 % of cases be greater than the reproducibility limit (R) values[2] given in **Table A.1** on average as long as the specified operation is definitely done[1].

10 Quality control

The laboratory is required to have internal quality control procedures for tests.

11 Test report

The test report shall include at least the following information:

a) a reference to this JAS standard;

b) identification of the sample;

c) the date of the test;

d) the results of the test.

Annex A
(informative)
Results of interlaboratory tests

Interlaboratory tests in accordance with IUPAC protocol[3] carried out in 2018 in Japan, gave the statistical results shown in **Tables A.1**. Commercially available or other provided tomatoes, which the calyx were removed, added the pyrogallol 3 % of sample mass and pureed.

After homogeneity [4] was confirmed, pureed samples were used as a test sample.

The experimental protocol and test samples were supplied to the participants by the Food and Agricultural Materials Inspection Center (FAMIC) organized this interlaboratory tests. All participants, respectively, tested a total of 12 test samples (6 pairs of blind duplicates) according to the experimental protocol.

NOTE Since lycopene may be decomposed by light, oxygen, enzymes contained in samples, antioxidants were added to stabilize the lycopene concentration of the test sample 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	38.6	45.6	61.9	97.2	119	169
Repeatability standard deviation s_r, mg/kg	0.48	0.67	1.2	2.7	2.2	5.1
Repeatability relative standard deviation, %	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, %	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 principle and definitions

NOTE The expression of the repeatability limit and reproducibility limit referred to section 7.1.5.

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

NOTE The calculation of the repeatability limit and reproducibility limit referred to section 4 “Determination of limits”.

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

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

NOTE The method of homogeneity referred to section 3.11 “Testing for sufficient homogeneity and stability”.

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

NOTE The absorption coefficient of lycopene referred to “MAIN LIST 31(Lycopene) Spectroscopic data”.