

codex alimentarius commission



FOOD AND AGRICULTURE
ORGANIZATION
OF THE UNITED NATIONS

WORLD
HEALTH
ORGANIZATION



JOINT OFFICE: Viale delle Terme di Caracalla 00153 ROME Tel: 39 06 57051 www.codexalimentarius.net Email: codex@fao.org Facsimile: 39 06 5705 4593

Agenda Item 5a)

CX/MAS 07/28/6

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

Twenty-eighth Session

Budapest, Hungary, 5 – 9 March 2007

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS IN CODEX STANDARDS

This document contains the Methods of analysis proposed by the following Committees in Draft Standards and Proposed Draft Standards under elaboration or as update of current methods.¹

This document includes the replies of individual Committees to the questions from the last session(s) of CCMAS concerning specific methods of analysis under the relevant sections.

No provisions for sampling have been sent for endorsement.

- A. Codex Committee on Processed Fruits and Vegetables
- B. FAO/WHO Coordinating Committee for Asia
- C. Codex Committee on Fish and Fishery Products

¹ Additional methods for endorsement will be presented separately (Committee on Nutrition and Foods for Special Dietary Uses and, if required, Committee on Fats and Oils).

A. CODEX COMMITTEE ON PROCESSED FRUITS AND VEGETABLES²

1. Draft Standard for Pickled Fruits and Vegetables (At Step 8)

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type	Status
Arsenic	AOAC 952.13 (Codex General Method)	Colorimetry, diethyldithiocarbamate			II	E
Arsenic	ISO 6634:1982	Spectrophotometry, silver diethyldithiocarbamate			III	E
Benzoic acid	AOAC 983.16	Gas Chromatography			II	E
Benzoic acid	NMKL 124 (1997)	Liquid Chromatography	The CCPFV should consider more modern methods (LC method) such as NMKL 124 (1997).	ADD - As recommended by CCMAS		
Benzoic acid	NMKL 103 (1984)	Gas Chromatography		DELETE	II	E
Drained weight	AOAC 968.30 (Codex General Method for processed fruits and vegetables)	Sieving Gravimetry	The CCMAS deleted this methods as no relevant provisions existed in the draft Standard. The CCMAS endorsed AOAC 968.30 as a general method for the determination of drained weight in processed fruits and vegetables. This method replaces CAC/RM 36/1970.	REINSERT	I	
Fill of containers	CAC/RM 46-1972 (Codex General Method for processed fruits and vegetables)	Weighing	The CCMAS retained the method while deleting the references to “metal containers” and refer to ISO 90:1:1986 for determination of water capacity in metal containers.	ADD	I	
Lead	AOAC 972.25 (Codex General Method)	Atomic absorption spectrophotometry			II	E

² ALINORM 07/30/27, Appendices II to V

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type	Status
Lead	ISO 6633:1984	Flameless atomic absorption spectrophotometry	The CCMAS recalled that the method proposed as Type IV for lead was temporarily endorsed since 1998 and asked the CCPFV whether this method was necessary since a general Codex method AOAC 972.25 already existed as Type II.	DELETE	IV	TE
pH	AOAC 981.12	Potentiometry		ADD	I	
pH	NMKL 179:2005		The CCMAS endorsed this method for the determination of pH in processed fruits and vegetables (except canned bamboo shoots)		II	
Sorbate	AOAC 983.16	Gas Chromatography			II	E
Sorbate	NMKL 124 (1997)	Liquid Chromatography	The CCPFV should consider more modern methods (LC method) such as NMKL 124 (1997).	ADD - As recommended by CCMAS		
Sorbate	NMKL 103 (1984)	Gas Chromatography		DELETE	II	E
Sulphur Dioxide	EN 1988-1:1998-02 AOAC 990.28 (sulphites)	Optimized Monier-Williams method	General method for sulphites (food additives)		III	E
Tin	AOAC 980.19 (Codex General Method)	Atomic absorption spectrophotometry			II	E
Tin ≤ 250.0 mg/kg	ISO 2447:1998	Spectrophotometry	The CCPFV should consider using the General Codex Method AOAC 980.19 and clarify why this method is proposed.	DELETE		NE

2. Draft Standard for Processed Tomato Concentrates (At Step 8)

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type	Status
Fill of containers	CAC/RM 46-1972 (Codex General Method for processed fruits and vegetables)	Weighing	The CCMAS retained the method while deleting the references to “metal containers” and refer to ISO 90:1:1986 for determination of water capacity in metal containers.	ADD	I	

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type	Status
Lactic Acid	EN 1139	Enzymatic determination	European Industry General Method for determination of Lactic acid in processed tomato concentrates	ADD		
Mineral impurities (sand)	AOAC 971.33 (Codex General Method for processed fruits and vegetables)	Gravimetry	The CCMAS endorsed AOAC 971.33 as a general method for the determination of mineral impurities in jams, jellies and marmalades and processed tomato concentrates. This method replaces CAC/RM 49/1972.		I	E
Mould count	AOAC 965.41	Howard mould count		ADD	I	
pH	AOAC 981.12	Potentiometry	The CCMAS endorsed this method for the determination of pH in processed fruits and vegetables (except canned bamboo shoots)	ADD	I	
pH	NMKL 179:2005				II	
Sodium Chloride	ISO 3634:1979 expressed as sodium chloride- (Codex General Method)	Potentiometry			III	E
Solids (soluble)	AOAC 932.12 ISO 2173:1978 (Codex General Method for processed fruits and vegetables)	Refractometry		ADD	I	
Tomato soluble solids	AOAC 970.59		AOAC 970.59 is already contained in CX/STAN 234/1999 for processed tomato concentrates.		I	E

3. Draft Standard for Preserved Tomatoes (At Step 8)

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type	Status
Calcium	AOAC 968.31 (Codex General Method for processed fruits and vegetables)	Complexometry Titrimetry	The CCMAS endorsed AOAC 968.31 as a general method for the determination of calcium in processed fruits and vegetables. This method replaces CAC/RM 38-1970.	ADD	II	
Drained weight	AOAC 968.30 (Codex General Method for processed fruits and vegetables)	Sieving Gravimetry	The CCMAS endorsed AOAC 968.30 as a general method for the determination of drained weight in processed fruits and vegetables. This method replaces CAC/RM 36/1970.	ADD	I	
	ISO UNIUN SERIES 2331	Sieving	For Crushed Style Only	NEW		
Fill of containers	CAC/RM 46-1972 (Codex General Method for processed fruits and vegetables)	Weighing	The CCMAS retained the method while deleting the references to “metal containers” and refer to ISO 90:1:1986 for determination of water capacity in metal containers.	ADD	I	
Mould count	AOAC 965.41	Howard mould count		ADD	I	
pH	AOAC 981.12	Potentiometry		NEW	I	
pH	NMKL 179:2005		The CCMAS endorsed this method for the determination of pH in processed fruits and vegetables (except canned bamboo shoots)	ADD	II	
Solids (Soluble)	AOAC 932.12 ISO 2173:1978 (Codex General Method for processed fruits and vegetables)	Refractometry		ADD	I	

4. Draft Standard for Certain Canned Citrus Fruits (At Step 8)

Provision	Method	Principle	Note	Recommendation CCPFV to CCMAS	Type proposed
Calcium	AOAC 968.31 (Codex General Method for processed fruits and vegetables)	Complexometry Titrimetry	The CCMAS endorsed AOAC 968.31 as a general method for the determination of calcium in processed fruits and vegetables. This method replaces CAC/RM 38-1970.	ADD	II
Drained weight	AOAC 968.30 (Codex General Method for processed fruits and vegetables)	Sieving Gravimetry	The CCMAS endorsed AOAC 968.30 as a general method for the determination of drained weight in processed fruits and vegetables. This method replaces CAC/RM 36/1970	ADD	I
Fill of containers	CAC/RM 46-1972 (Codex General Method for processed fruits and vegetables)	Weighing	The CCMAS retained the method while deleting the references to “metal containers” and refer to ISO 90:1:1986 for determination of water capacity in metal containers.	ADD	I
Solids (Soluble)	AOAC 932.12 ISO 2173:1978	Refractometry	Codex General Method for processed fruits and vegetables	ADD	I

B. FAO/WHO COORDINATING COMMITTEE FOR ASIA³

Proposed Draft Standard for Gochujang (At Step 5)

COMMODITY	PROVISION	METHOD	PRINCIPLE
Gochujang	Capsaicin	According to the method described in the Annex A or B.	
	Crude Protein	AOAC 984.13 (Nitrogen conversion factor: 6.25).	Kjeldahl
	Moisture	AOAC 934.01.	Gravimetry

Annex A

Determination of capsaicin in *Gochujang* using GC detection

1. SCOPE

This method is suitable for the determination of capsaicin and dihydrocapsaicin (DHC) in *Gochujang* using chromatographic detection. The method uses squalene as an internal standard. The concentration of capsaicin and dihydrocapsaicin is expressed as ppm.

2. PRINCIPLE

To extract capsaicin and DHC, the mixture is blended to a homogeneous consistency. Capsaicin in *Gochujang* is extracted with 100% methanol, followed by methanol – hexane fractionation to remove hydrophilic and hydrophobic interfering substances by a separating funnel. Capsaicin in methanol layer is extracted with dichloromethane (DCM) and the saturated NaCl, concentrated by a rotary evaporator. A portion of the concentrated sample extract is then taken and completely solved with DCM containing squalene as an internal standard for analysis using gas chromatographic detection.

3. REAGENT AND MATERIALS

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade and water of at least grade 3 as defined in ISO 3696.

3.1 Reagents

- 3.1.1 Capsaicin (99 + %, C₁₈H₂₇NO₃, Fw 305.42, CAS 404-86-4)
- 3.1.2 Dihydrocapsaicin (90 + %, C₁₈H₂₉NO₃, Fw 307.42, CAS 19408-84-5)
- 3.1.3 Squalene (CAS 111-02-4)
- 3.1.4 Hexane
- 3.1.5 Methanol
- 3.1.6 Methanol + Water (80 + 20)
- 3.1.7 Dichloromethane
- 3.1.8 Sodium chloride
- 3.1.9 Sodium sulfate

3.2. Preparation of standard solution

3.2.1 Capsaicin Stock solution (A)

Weigh approximately 100 mg of each capsaicin and DHC, making up to 100 mL in a volumetric flask with DCM to give solution (A) of approximate 1000 µg/mL.

3.2.2 Capsaicin working solution (B)

Prepare 100 mL intermediate solution B by dilution of 10 mL solution A (3.2.1) with 100 mL of DCM to exactly 100 µg/mL in DCM.

³ ALINORM 07/30/15, Appendix II

3.2.3 Squalene internal standard working solution (C)

Weigh approximately 100 mg squalene and make up to 250 mL in a volumetric flask with DCM to give a solution (C) of approximately 400 µg/mL in DCM.

3.3 Calibration solutions of capsaicin

Dispense volumes of the 100 µg/mL solution (B, 3.2.2) into 50 mL round flask, dried up and add 2 mL of internal standard working solution (C, 3.2.3) to give 10.0, 50.0, 100.0, 300.0, 500.0 µg/mL capsaicin.

4. APPARATUS

4.1 Gas chromatograph with flame ionization detector (FID)

The following conditions have been found to be suitable:

4.1.1 Injector / Detector temperature : 320°C / 350°C

4.1.2 Oven temperature program: 220°C for 1 minute, ramp at 5°C/min to 250°C, hold for 13 minutes and raise to 280°C holding 5 min by 20°C/min. Helium carrier gas at 1.5 mL/minute

4.1.3 Make split injection of 1.0 µL with split ratio 1:5

4.2 GC column, 30 m x 0.32 µm, 0.25 µm film thickness, HP-1 or equivalent

4.3 Analytical balance, capable of weighing to 4 decimal places

4.4 Shaker, capable of attaining 2,000 rpm

4.5 Centrifuge, capable of attaining 3,500 rpm

4.6 Filter paper (Waterman No. 2 or equivalent)

5. LABORATORY SAMPLES

On receipt, samples are given a unique sample number. *Gochujang* sample is stored at below 4°C. All other samples are stored at room temperature in an air tight container prior to analysis.

6. PROCEDURE

6.1 Laboratory sample

Samples should be minced or grated to a homogeneous mixture. All samples should be stored in the air-tight container and at room temperature prior to analysis. All samples should be mixed thoroughly to a homogeneous mixture before analysis.

6.2 Test sample

6.2.1. Thoroughly mix the sample. Weigh, to the nearest 0.01 g, and 10 g portion of *Gochujang* into a centrifuge bottle (250 mL, Nalgene).

6.2.2 Add 50 mL of methanol and shaking for 2 hours, extracting capsaicin.

6.2.3 Filter the extract with Watman No. 2 filter paper into a 250 mL flask (Ext-A).

6.2.4 Add additional 30 mL of methanol to residue and shaking for 1 hour, extracting capsaicin (Ext-B).

6.2.5 Repeat step 6.2.3 to 6.2.4 (Ext-C)

6.2.6 Combine Ext-A, Ext-B and Ext-C in 250 mL round bottom flask, concentrating up to approximately 5 mL.

6.2.7 Solve the concentrate with 20 mL of 80% methanol and 20 mL of hexane.

6.2.8 Transfer the solution into a 250 mL separating funnel.

6.2.9 Shake and separate into two layers, methanol layer (M1-layer, upper) and hexane layer (H1-layer, lower)

6.2.10 Reserve H1-layer in 100mL flask and transfer M1-layer (6.2.9) into a separating funnel and add additional 20 mL of hexane.

- 6.2.11 Repeat step 6.2.9 to 6.2.10 (M2-layer and H2-layer)
- 6.2.12 Repeat step 6.2.9 to 6.2.10 (M3-layer and H3-layer)
- 6.2.13 Combine H1-layer, H2-layer and H3-layer (HC-layer) in the 250mL separating funnel, adding 20 ml 80% methanol, shaking and separating into two layers, methanol layer (M'1-lower layer) and hexane layer (H'1-upper layer).
- 6.2.14 Reserve M'1-layer in the new 250 mL flask.
- 6.2.15 Add 20 mL of 80% methanol into the separating funnel containing HC-layer, shaking and separating into two layer (M'2-layer and H'2-layer)
- 6.2.16 Combine the all M-layer in the new separating funnel (250 mL), adding 20 mL of saturated NaCl and 20 mL of DCM.
- 6.2.17 Shake and separate into two layer (D1-layer and WM1-layer) in the 250 mL separating funnel.
- 6.2.18 Transfer D1-layer into the new 250 mL round flask.
- 6.2.19 Add additional 20 mL DCM into the separating funnel (6.2.16), shaking and separating into two layers (D2-layer and WM1-layer)
- 6.2.20 Repeat step 6.2.16 (D3-layer and WM1-layer)
- 6.2.21 Combine D1-layer, D2-layer and D3-layer into the 250 round flask, concentrating it (C-D)
- 6.2.22 Transfer the concentrate (C-D, 6.2.21) into a 100 mL round flask, solving it completely with DCM.
- 6.2.23 Mount approximate 3 g of sodium sulfate on the filter paper and dehydrate C-D by passing through sodium sulfate
- 6.2.24 Collect the dehydrated C-D layer in 50 mL round flask and concentrate to dryness by the rotary evaporator
- 6.2.25 Solve the concentrate with 2 mL of DCM containing squalene as the internal standard solution (C, 3.2.3)
- 6.2.26 Analyze the sample solution by GC

7. CALCULATION – INTERNAL STANDARD METHOD

- 7.1** Measure the area of the capsaicin and squalene peaks.
- 7.2** Calculate the ratio of the capsaicin and squalene peak areas.
- 7.3** Construct a calibration graph for the standards by plotting the peak area ratio against the weight in microgram of capsaicin in the vial.
- 7.4** Calculate the slope of the calibration line.
- 7.5** Divide the peak area ratio of the unknowns by the value of the slope to give the weight of capsaicin per vial for the unknown samples.

8. FINAL PRESENTATION OF RESULTS

Results are expressed as ppm and quoted to 2 significant digits.

REFERENCES

1. W. Hawer and J. Ha et al. : Effective separation and quantitative analysis of major heat principles in red pepper by capillary GC, Food chemistry, 49, pp.99-103, 1994.
2. J. Jung and S. Kang : A new method for analysis of capsaicinoids content in microcapsule, Korean J. Food Sci. Technol., Vol.32, No. 1, pp.42-49, 2000.
3. C.A. Reilly et al. : Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products, J. of Forensic science, Vol.43, No. 3, ppp.502-509, 2001.

Appendix I.

Table 1. Summary of repeatability test for trial proper samples (ppm)

Test No.	<i>Gochujang - K</i>	
	CAP	DHC
1	64.7	55.4
2	69.0	51.4
3	70.6	53.5
4	71.8	52.3
5	70.5	52.4
Mean	69.3	53.0
RSD,%	3.99	2.90

Table 2. Summary of recovery test for trial proper samples (%)

Test No.	<i>Gochujang - K</i>	
	CAP	DHC
1	80.47	74.53
2	77.29	78.68
3	87.97	85.09
4	91.00	89.25
5	95.18	90.38
Mean	86.38	83.58
RSD,%	8.56	8.17

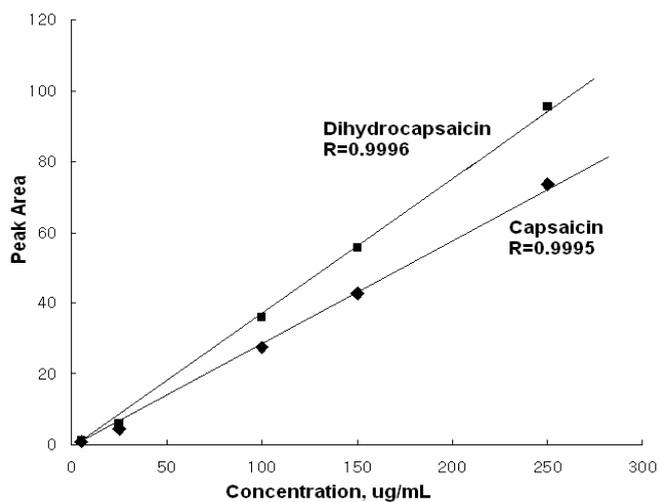


Fig.1. Calibration curve of capsaicin and DHC by GC method.

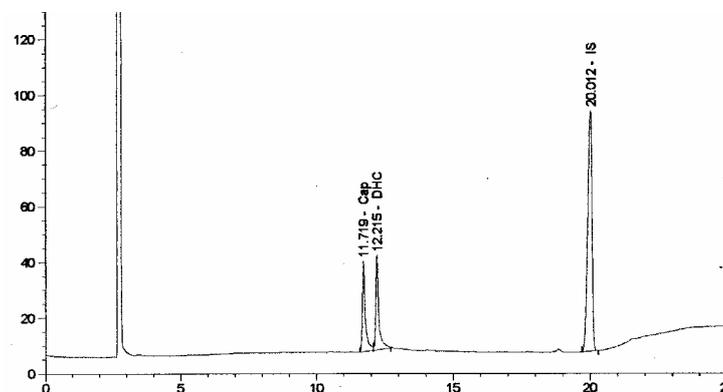


Fig. 2. GC chromatogram of capsaicin standards.

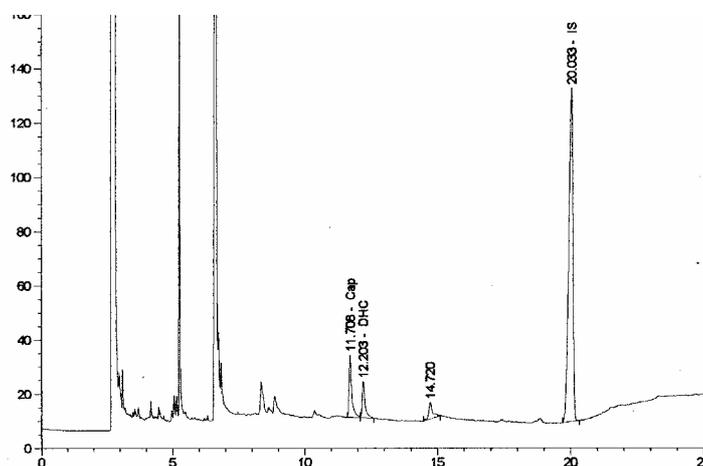


Fig. 3. GC chromatogram of capsaicin in *Gochujang*.

Determination of capsaicin in *Gochujang* using HPLC detection

1. SCOPE

This method is suitable for the determination of capsaicin and dihydrocapsaicin in *Gochujang* using liquid chromatographic detection. The concentration of capsaicin is expressed as ppm.

2. PRINCIPLE

To extract capsaicin, the mixture is blended to a homogeneous consistency. Mixture solution (NaOH and NaCl) is added to the sample and capsaicin in sample is extracted with hexane and diethyl ether. A portion of the concentrated sample extract in methanol is then taken for analysis using liquid chromatographic detection.

3. REAGENTS AND MATERIALS

During the analysis, unless otherwise stated, use only reagent of recognized analytical grade and water of at least grade 3 as defined in ISO 3696.

3.1 Reagents

3.1.1 Capsaicin (99.0 + %, $C_{18}H_{27}NO_3$, Fw 305.42, CAS 404-86-4)

3.1.2 Dihydrocapsaicin (99.0 + %, $C_{18}H_{29}NO_3$, Fw 307.42, CAS 19408-84-5)

3.1.3 Hexane

3.1.4 Diethyl ether

3.1.5 Methanol

3.1.6 Sodium perchlorate

3.1.7 Sodium hydroxide

3.1.8 Sodium chloride

3.1.9 Sodium sulfate

3.2 Preparation of standard solutions

3.2.1 Capsaicin stock solution (A)

Weight approximately 10 mg of capsaicin and dihydrocapsaicin and make up to 20 mL in a volumetric flask with methanol to give solution (A) of approximate 500 $\mu\text{g/mL}$.

3.2.2 Capsaicin calibration solution

The 500 $\mu\text{g/mL}$ solution (A, 3.2.1) diluted to give the concentration of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 $\mu\text{g/mL}$ capsaicin in methanol.

3.3 Extracting solutions

3.3.1 Hexane : Diethyl ether solution (C)

Blend 70 portion of hexane and 30 portion of diethyl ether, producing 70:30 solution C

3.3.2 0.5 mol/L NaOH + 2% NaCl solution (D)

Dissolve 2 g of NaCl in 100 mL of 0.5 mol/L NaOH to give the concentration of 2% (w/v).

4. APPARATUS

4.1 Liquid chromatograph with fluorescence detector

4.2 Mobile phase : Methanol : 0.1 mol/L NaClO_4 = 6:4 (v/v)

4.3 HPLC column : Mightysil RP18GP (5 μm x 4.6 mm x 15 cm, Kanto Chemical Co.) or equivalent

4.5 Detector : Fluorescence, Ex. 283 nm, Em. 316 nm

4.6 Filter paper (Waterman no. 4 or equivalent)

4.7 Analytical balance, capable of weighing to 4 decimal places

4.8 Centrifuge, capable of attaining 3,500 rpm

4.9 pH meter

5. LABORATORY SAMPLES

On receipt, samples are given a unique sample number. Samples contained a large quantity of water are stored at below 4°C. All other samples are stored at room temperature in an air tight container prior to analysis.

6. PROCEDURE

6.1 Laboratory sample

Samples should be minced or grated to a homogeneous mixture. All Samples should be stored in an air-tight container and at room temperature prior to analysis. All samples should be mixed thoroughly to a homogeneous mixture before analysis.

6.2 Test sample

6.2.1. Thoroughly mix the sample. Weigh 2 g of *Gochujang*, to the nearest 0.01 g, into a centrifuging bottle (250 mL, Nalgene).

6.2.2 Add 15mL of hexane/diethylether 7:3 solution (v/v, C, 3.3.1) and 30 mL of 0.5 mol/L NaOH + 2% NaCl (D, 3.3.2), mincing sufficiently to extract capsaicin.

6.2.3 Shaking for 10 minutes, centrifuging for 5 minutes at 2,000 rpm.

6.2.4 Transfer lower layer (L1) into the new centrifuging bottle (250 mL) and reserve it.

6.2.5 Add additional 20 mL of 0.5 mol/L NaOH + 2% NaCl (D, 3.3.2) into the original bottle containing the upper layer.

6.2.6 Repeat 6.2.3 to 6.2.4 and collect L1, L2 and L3 into the new centrifuging bottle.

6.2.7 Adjust pH 2.0 with conc. HCl

6.2.8 Add 80 mL of hexane/ether (C, 3.3.1) and shaking for 5 minutes.

6.2.9 Collect upper layer (U1) into 300 mL round flask using a pipette.

6.2.10 Repeat 6.2.8 to 6.2.9 and collect upper layer U2.

6.2.11 Transfer U1 and U2 into 300 mL round flask (Ext-U)

6.2.12 Mount approximate 3 g of sodium sulfate on the filter paper and dehydrate Ext-U by passing through sodium sulfate

6.2.13 Collect the dehydrated Ext-U in a 300 mL round flask and concentrate to dryness by the rotary evaporator

6.2.14 Solve the concentrate completely with methanol and fill up in a volumetric flask with 10 mL of methanol

6.2.15 Analyze the sample solution by HPLC

7. CALCULATION - EXTERNAL STANDARD METHOD

7.1 Measure the height of the capsaicin peaks.

7.2 Construct a calibration graph for the standards by plotting the peak area ratio against the weight in microgram of capsaicin in the vial.

7.3 Calculate the slope of the calibration line.

7.4 Divide the peak area ratio of the unknowns by the value of the slope to give the weight of capsaicin per vial for the unknown samples.

8. FINAL PRESENTATION OF RESULTS

Results are expressed as ppm and quoted to 3 significant digits.

REFERENCES

1. W. Hawer and J. Ha et al. : Effective separation and quantitative analysis of major heat principles in red pepper by capillary GC, Food chemistry, 49, pp.99-103, 1994.
2. J. Jung and S. Kang : A new method for analysis of capsaicinoids content in microcapsule, Korean J. Food Sci. Technol., Vol.32, No. 1, pp.42-49, 2000.
3. Christopher A. Reilly, Dennis J. Crouch and Garold S. Yost : Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products, J. Forensic Science 46(3), 502-509, 2001.

Appendix I.

Table 1. Summary of repeatability test for trial proper samples (ppm)

Test No.	<i>Gochujang - J</i>	
	CAP	DHC
1	42.0	17.2
2	41.6	17.1
3	41.0	16.5
4	40.2	17.2
5	40.7	17.5
Mean	41.1	17.1
RSD,%	1.73	2.14

Table 2. Summary of recovery test for trial proper samples (%)

Test No.	<i>Gochujang - J</i>	
	CAP	DHC
1	95.5	98.1
2	99.0	99.2
3	98.3	97.0
4	97.8	97.4
5	101.9	101.6
6	95.6	96.0
Mean	98.0	98.2
RSD,%	2.4	2.0

Appendix II.

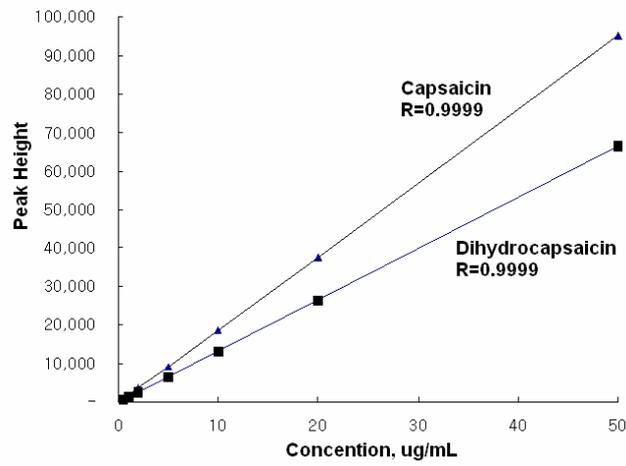


Fig. 1. Calibration curve of capsaicin and DHC by HPLC method.

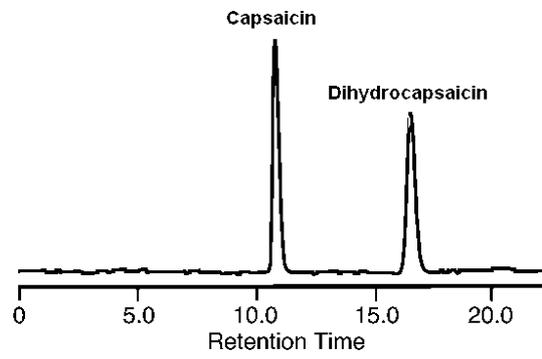


Fig. 2. HPLC chromatogram of standard capsaicin.

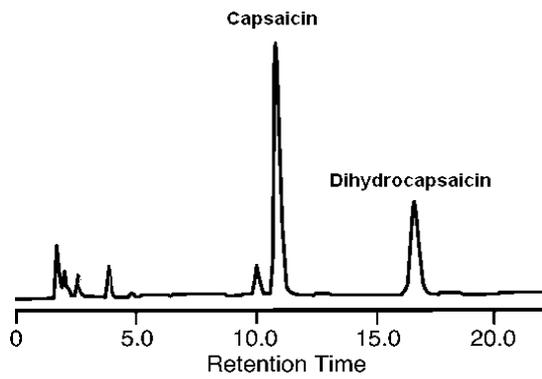


Fig. 3. HPLC chromatogram of capsaicin in *Gochujang*.

C. CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS⁴

Proposed Draft Standard for Live and Raw Bivalve Molluscs (At Step 5)

COMMODITY	PROVISION	METHOD	PRINCIPLE
Raw Bivalve Molluscs	Net weight of products covered by glaze	AOAC 963.18	weighing
	Net weight of products covered by glaze with water added inside a "block-frozen" product	AOAC 963.26	weighing
	Drained weight of shucked molluscs	AOAC 953.11	weighing

Determination of Biotoxins

The majority of the currently available methods do not meet all Codex criteria for reference methods (Type II). There are a number of chemical methods, instrumental methods and functional assays currently in use. These are listed in the table below.

<i>Provision</i>	<i>Methodology</i>	<i>Principle</i>	<i>Type</i>
<i>Saxitoxin Group</i>	<i>AOAC Official Method 2005.06 (Paralytic Shellfish Poisoning Toxins in Shellfish)</i>	<i>LC-FL</i>	<i>II</i>
	<i>AOAC International Mouse Bioassay</i>	<i>Bioassay</i>	<i>III</i>
	*	<i>Receptor Binding Assay</i>	<i>III</i>
	*	<i>Immunochemical</i>	<i>III</i>
	*	<i>LC-MS²</i>	<i>III</i>
<i>Okadaic Acid Group</i>	*	<i>LC-MS²</i>	<i>II</i>
	*	<i>Bioassay^{1,2}</i>	<i>III</i>
	*	<i>PP2A²</i>	<i>III</i>
	*	<i>LC-FL</i>	<i>III</i>
	*	<i>ELISA²</i>	<i>III</i>
<i>Domoic Acid Group</i>	<i>Quilliam LC-UVD method</i>	<i>LC-UV</i>	<i>II</i>
	*	<i>ELISA</i>	<i>III</i>
	*	<i>LC-MS</i>	<i>III</i>
	*	<i>LFIC²</i>	<i>III</i>
<i>Brevetoxin Group</i>	*	<i>LC-MS²</i>	<i>II</i>
	*	<i>ELISA²</i>	<i>III</i>
	<i>APHA mouse bioassay</i>	<i>bioassay¹</i>	<i>III</i>
<i>Azaspiracid Group</i>	*	<i>LC-MS²</i>	<i>II</i>
	*	<i>bioassay¹</i>	<i>III</i>

⁴ ALINORM 07/30/18, Appendix V

¹ When using the MBA for detecting lipophilic marine biotoxins, false positives may occur due to the presence of other substances such as YTX, PTX and CI, which are not known to cause human illness. When false positives are suspected, confirmatory testing, using an internationally validated method, can be carried out in order to identify the type(s) of marine biotoxins present.

² Further method development (e.g. interlaboratory validation, CRM availability) needed prior to submission for endorsement by CCMAS

* Official /recognized method title to be identified

Note: Only the methods corresponding to the requirements for endorsement are for consideration by the Committee. The other methods are listed for information as to the status of methodology in this area.