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World Health Organization

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PROPOSED DRAFT REGIONAL STANDARD FOR SOYBEAN PRODUCTS FERMENTED WITH *BACILLUS* SPECIES

(at Step 4)

(Prepared by the Electronic Working Group chaired by Japan)

Codex Members and Observers wishing to submit comments, at Step 3, on this proposed draft (Appendix I) should do so as instructed in CL 2022/52/OCS-ASIA available on the Codex webpage/Circular Letters 2022

BACKGROUND

1. At the 19th session of the FAO/WHO Coordinating Committee for Asia (CCASIA19) (November 2014)[1], Japan submitted a discussion paper proposing a new work on the development of a regional standard for *Natto*, a Japanese traditional fermented soybean food. CCASIA19 agreed that Japan would revise the discussion paper to include information on similar products in the region, the possibility to revise existing standard to include *Natto*, and a justification for the development of the standard.

2. At CCASIA20 (September 2016)[2], Japan proposed to expand the scope of the new work proposal from the single commodity "Natto" to "soybean products fermented with the bacterium *Bacillus subtilis*" in order to develop a more overarching standard. CCASIA20 agreed to request Japan to further revise the discussion paper and the project document for new work, with the assistance of interested CCASIA members, and to clearly provide the information listed in the "Criteria for establishment of work priorities" such as impediments to trade, diversification of national legislation and amenability to standardization.

3. Following CCASIA20, Japan convened an informal group and had discussions among interested members so as to make the scope of the standard as broad as possible. At CCASIA21[3] (September 2019), taking into account all comments/information provided by interested members, Japan proposed to expand the scope of the new work proposal to soybean products fermented with *Bacillus* species other than *Bacillus subtilis*, to ensure a more inclusive regional standard. CCASIA21 agreed to forward the project document to the 43rd Session of the Codex Alimentarius Commission (CAC43) for approval as new work and establish an EWG chaired by Japan, working in English, and subject to the approval of the new work, to prepare the proposed draft standard for circulation for comments at Step 3 and consideration at CCASIA22.

4. CAC43[4] (September to November 2020) approved the new work proposal on development of a regional standard for soybean products fermented with *Bacillus* species.

TERMS OF REFERENCE

5. CCASIA21 agreed to establish an EWG chaired by Japan, working in English, subject to the approval of the new work, to prepare the proposed draft standard for circulation for comments at Step 3 and consideration at CCASIA22.

[1] REP15/ASIA paragraph 118
[2] REP17/ASIA paragraph 111
[3] REP17/ASIA paragraph 97(ii)
[4] REP20/CAC paragraph 54 and Appendix V

PARTICIPATION AND METHODOLOGY

6. The invitation to participate in the EWG was circulated to Codex members and observers in June 2020. Seven member countries and three observer countries participated in the EWG. The EWG Chair re-sent the invitation to join the EWG to remaining member countries that produce a product covered by the scope of the project document, but received no response. The list of participants is attached as Appendix II. The work was undertaken on the EWG online platform.

7. Due to the COVID-19 global pandemic, CCASIA22 was not held in 2021 as anticipated; however, taking advantage of the additional time, the EWG continued to progress the work through three rounds of discussions. The 1st draft was posted in December 2020 and comments were submitted by China, the Republic of Korea, and Nigeria who was an observer country. The 2nd draft was posted in August 2021 and comments were submitted by the Republic of Korea, Thailand and Nigeria who was an observer country. The 3rd draft was posted in February 2022 and comments were submitted by the Republic of Korea and Thailand. In addition, the data related to the methods of analysis were provided by Japan and the Republic of Korea to support development of the standard.

SUMMARY OF DISCUSSION

8. The comments received during three rounds of discussions, in particular on the specific products, were considered and reflected in the draft to a large extent. Editorial comments or comments proposing clarifying wording were also considered and many of those comments were reflected in the draft to improve clarity. Analysis for the main proposed changes and those justifications are indicated in the paragraphs below:

1. Scope and 2.2. Classification

9. In the project document of this work, the products to which the standard would apply included *Natto*, *Cheonggukjang, Douchi, Kinema* and *Tua Nao Sa*. During the first round of the discussion, China, who is the main producer of *Douchi*, commented that most *Douchi* are primarily fermented by fungi, and rarely by bacteria (*Bacillus* spp.). As a result, *Douchi* was excluded from 2.2 Classification. During the second round of the discussion, Thailand, who is the main producer of *Tua Nao Sa*, requested to change the name of "*Tua Nao Sa*" to "*Tua Nao*" to cover all types of *Thua Nao* and their derived product. Therefore, the name was changed to "*Tua Nao*".

3. Essential Composition and Quality Factors

10. *Kinema* was also one of the products to which the standard applied in the project document of this work. However, information on *Kinema* was not provided to EWG during three rounds of discussions. Therefore, the EWG could not draft any specific provisions for *Kinema* in 3. Essential Composition and Quality Factors.

3.1.1 Basic Ingredients

11. CCASIA21 noted the concern on the use of the term *Bacillus* spp. as some species could be considered pathogenic and agreed to take this concern into consideration in the development of the standard. The EWG agreed to include "these are not pathogenic and do not produce toxins." in 3.1.1 (c) *Bacillus* spp. (Naturally occurring or cultivated microorganisms), as the sentence is used in *Regional Standard for Fermented Soybean Paste* (CXS 298R-2009).

7.3. Labelling of Non-Retail Containers

12. CAC44 (November 2022) adopted *General Standard for the Labelling of Non-retail Containers of Foods* (CXS 346-2021). The reference to this standard was added to the third draft of EWG. There were no comments on this from members during the 3rd round of the discussion.

8. Methods of Analysis and Sampling

13. Japan provided validation results of the methods of analysis for *Natto* to the EWG during the second round of the discussion., There were no comments from members on these results, The validation results of the methods of analysis for *Natto* are attached as Annex I of Appendix I. For *Cheonggukjang*, the Republic of Korea provided validation results of the methods of analysis during the third round of the discussion, but the EWG members did not have enough time to review these results. The validation results of the methods of analysis for *Cheonggukjang* are attached as Annex II of Appendix I.

Weights and Measures and Lot Acceptance

14. CCASIA21 agreed not to include "weights and measures", in the project document but to consider whether or not this provision was needed when drafting the standard. during the second and third round of the discussion, Thailand proposed to include "Weights and Measures" and "Lot Acceptance" in the standard. The EWG chair has reflected the proposal in the proposed draft standard but recognized that containers may widely

vary between products and sometimes weight could change during the fermentation and storage. The EWG chair would like to recommend that CCASIA22 consider the contents of these provisions.

CONCLUSION AND RECOMMENDATION

15. In view that almost all the submitted comments were addressed, the EWG chair considers that the proposed draft standard is ready to be submitted to CCASIA22 for consideration at Step 4. CCASIA22 is invited to consider the proposed draft standard (Appendix I), with a view to progress it through the Codex step procedure.

Appendix I

PROPOSED DRAFT REGIONAL STANDARD FOR SOYBEAN PRODUCTS FERMENTED WITH *BACILLUS* SPECIES

1. SCOPE

This standard applies to products, as defined in Section 2, for direct consumption, including for catering purposes, repacking or further processing if required. This standard does not apply to the product covered by the *Regional Standard for Fermented Soybean Paste* (CXS 298R-2009).

2. DESCRIPTION

2.1. Product Definition

Soybeans fermented with *Bacillus* spp. (solely or together with other microorganisms), that normally retain the shape of soybeans and are not a type of paste, although some of the soybeans may be crushed during the manufacturing process. The final products maybe sticky and could display various forms of products.

2.2. Classification

2.2.1. Natto

Soybeans (including crushed soybeans, hereinafter referred to as soybeans) are soaked in water or dilute salty water, then steamed and fermented with *Bacillus subtilis* var. *natto*. No materials shall be added after fermentation.

Stickiness

Natto shall be sticky and visible filamentous substance must be produced when a bean in *Natto* is picked up.

2.2.2. Cheonggukjang

Soybeans soaked in water are boiled, steamed or baked and then fermented with naturally occurring or cultivated microorganisms (i.e. *Bacillus* spp. including *Bacillus subtilis*) for a few days. Optional ingredients described in Section 3.1.2.2, may be added. The powder, paste and spherical pellet type of the products shall be additionally permitted provided that it meets all requirements of this standard.

Stickiness

Various viscous (i.e. filamentous) substance may be visible when *Cheonggukjang* is lifted.

2.2.3. Kinema

Soybeans are crushed lightly, after steamed, wrapped in broad leaves such as banana leave and fermented without spraying inoculums. After that, it may be dried in the sun. Furthermore, other microorganisms i.e. *Enterococcus*, *Candida*, *Geotrichum* may be contained.

Stickiness

Thin filamentous substance may be visible when *Kinema* is lifted.

2.2.4. Thua Nao

Soybeans are steamed or boiled, wrapped in broad leaves such as banana leave. They are fermented with *Bacillus* spp. (solely or may contain other microorganisms).

Stickiness

Thin filamentous substance may be produced.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1. Composition

3.1.1. Basic Ingredients

(a) Soybeans

(b) Potable water

(c) *Bacillus* spp. (Naturally occurring or cultivated microorganisms). These are not pathogenic and do not produce toxins.

3.1.2. Optional Ingredients

3.1.2.1. Natto

(a) Grains and/or flour (wheat, rice, barley, etc.)

(b) Salt

(c) Seaweed and/or seaweed powder

(d) Other ingredients as appropriate

3.1.2.2. Cheonggukjang

(a) Naturally occurring or cultivated microorganisms (other than *Bacillus* spp.). These are not pathogenic and do not produce toxins.

(b) Salt

(c) Garlic

(d) Red pepper powder

(e) Other ingredients as appropriate

3.1.2.3. Thua Nao

(a) Other naturally occurring or cultivated microorganisms (other than *Bacillus* spp.). These are not pathogenic and do not produce toxins

(b) Salt

(c) Other ingredients as appropriate

3.2. Quality Criteria

The soybean products fermented with *Bacillus* spp. shall have the characteristic flavour, odour, colour, and texture of the product. There should be no visible foreign matters in the products.

3.3. Component Requirement

The soybean products fermented with *Bacillus* spp. should comply with the composition requirements listed in Table 1.

Table 1 Composition (wet weight basis)

Product name	Moisture content (%,w/w)	Protein (%,w/w)	Lipid (%,w/w)
Natto	≥53.0	≥10.0	≥5.0
Cheonggukjang	≤58.0 (in case of powder type ≤15.0)	≥12.5	≥4.0
Thua Nao	≥53.0	≥10.0	—

3.4. Classification of "Defectives"

Any products that fails to meet the applicable quality requirements, as set out in Section 3.2, 3.3 and [3.4], should be considered a "defective".

3.5. Lot Acceptance

[A lot should be considered as meeting the applicable quality requirements referred to in Section 3.2, when the number of "defectives", as defined in Section 3.4, does not exceed the acceptance number (c) of the appropriate sampling plans.(TBD)]

4. FOOD ADDITIVES

None permitted.

5. CONTAMINANTS

5.1. The products covered by this standard shall comply with the Maximum Levels of the *General Standard for Contaminants and Toxins in Food and Feed* (CXS 193-1995).

5.2. The products covered by this standard shall comply with the maximum residue limits (MRLs) for pesticides established by the Codex Alimentarius Commission.

6. HYGIENE

It is recommended that the products covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the *General Principles of Food Hygiene* (CXC 1-1969), and other relevant Codex texts, such as Codes of Hygienic Practice and Codes of Practice.

The products should comply with any microbiological criteria established in accordance with the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria related to Foods* (CXG 21-1997).

7. Weights and measures

7.1. Fill of Container

7.1.1. Net weight

[The container should be filled with product without impairment of quality and shall be consistent with a proper declaration of contents for the product.]

7.1.2 Classification of "Defectives"

[A product that fails to meet the requirement of Section 7.1.1 should be considered as a "defective".]

7.1.3 Lot Acceptance

[A lot should be considered as meeting the requirements of Section 7.1.1 when the number of "defectives", as defined in Section 7.1.2 does not exceed the number (c) of the appropriate sampling plan.]

8. LABELLING

8.1. The products covered by the provisions of this standard shall be labelled in accordance with the *General Standard for the Labelling of Prepackaged Foods* (CXS 1-1985).

8.2. The Name of the Product

The products are soybean products fermented with *Bacillus* spp. The product should be designated with the appropriate term in Section 2.2. Other names may be used in accordance with the law and custom of the country of retail sale in the manner not to mislead consumers.

8.3. Labelling of Non-Retail Containers

The labelling of non-retail containers should be in accordance with the *General Standard for the Labelling of Non-Retail Containers of Foods* (CXS 346-2021).

9. METHODS OF ANALYSIS AND SAMPLING[5]

For checking the compliance with this standard, the methods of analysis and sampling contained in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) relevant to the provisions in this standard, shall be used.

9.1. Determination of Moisture Content

Natto: According to AOAC 925.09.(Type I)

Cheonggukjang: According to AOAC 934.01. (Type I)

Thua Nao: According to AOAC 925.09. (Type I)

9.2. Determination of Protein Content

Natto: According to AOAC 988.05. (Type I)

(Nitrogen factor 5.71)

[5] The testing methods will be removed when the standard is adopted by CAC and included in CXS 234-1999.

Cheonggukjang: According to AOAC 988.05. (Type I)

(Nitrogen factor 5.71)

Thua Nao: According to AOAC 988.05. (Type I)

(Nitrogen factor 5.71)

9.3. Determination of Lipid Content

Natto: According to AOAC 963.15. (Type I)

(Quantity of sample:4g)

Cheonggukjang: According to AOAC 963.15. (Type I)

(Quantity of sample:5g)

Annex I

Validation studies for methods of analysis for *Natto*

(submitted by Japan)

Background

At CCASIA 21, the Committee had agreed to start to work of Regional Standard for Soybean Products Fermented with *Bacillus* species and the proposal was approved by the CAC43.

In the proposed draft, soybean products fermented with *Bacillus* species may be classified as described in Section 2.2, for each of which quality criteria are established in Section 3. For classification, provisions for moisture, protein and lipid are listed and AOAC 925.09, AOAC 988.05 and AOAC 963.15, respectively, are proposed as analytical methods.

These three methods have already been endorsed as Type I for some soybean products – AOAC 925.09 for moisture content in soy protein products, tempe (produced by fermentation of soy bean with different microorganism from *Bacillus* species) and non-fermented soybean products, AOAC 988.05 for protein content in tempe and non-fermented soybean products, and AOAC 963.15 for lipid content in tempe . In particular, these three methods are all endorsed as Type I for tempe, whose PFC ratio is similar to that of *natto* (Table 1). Therefore, it is likely that these methods are also valid for *natto*.

Table 1 PFC ratios for *natto* and tempe, both of which were fermented products of soy bean

	Protein(%)	Fat(%)	Carbohydrate(%)
Natto	16.5	10.0	12.1
Tempe	15.8	9.0	15.4

Cited from Standard Tables of Food Composition in Japan 2020

To ensure applicability of these methods for *natto*, a single-laboratory validation study was conducted by Japan, understanding that CCMAS 41 had agreed that if the study represents an extension to a new matrix of a previously validated method, CCMAS does not require full collaborative study.

Validation study

The validation study was conducted at Japan Food Research Laboratories. Each analysis was conducted 7 times per day for 3 days.

Preparative procedure of a sample

Each sample was placed in a plastic bag and roughly crushed from the top of the bag using a mallet. Furthermore, the coarsely crushed product was crushed and homogenized with a food processor (DLC-N7J) [Conair Japan GK] for about 30 seconds. It was stored refrigerated (2 ° C to 8 ° C) until it was subjected to the test. For the two samples in each analysis, natto near the average value and the lower limit value of the components were selected and used for the analysis.

(1) Method of Moisture content analysis

Moisture content in two samples (sample A and B) were analyzed using AOAC925.09. Since a metal dish diameter ca 55 mm and height ca 15 mm is specified in AOAC 925.09, a metal dish diameter 55 mm and height 25 mm was used.

The results for sample A and B are shown in Table 2 and Table 3, respectively.

Table 2 Analyses of moisture content in sample A (3 days x 7 trials)

Trial	Moisture (g/100 g).		
	1st day	2nd day	3rd day
1	60.17	60.05	60.00
2	60.12	59.92	60.05

3	60.00	60.08	60.07
4	60.00	59.97	60.08
5	59.92	59.93	59.88
6	59.97	59.95	60.00
7	59.98	59.98	60.00
Repeatability relative standard deviation (%)	0.13		
Intermediate relative standard deviation (%) (intermediate precision)	0.13		

Table 3 Analyses of moisture content in sample B (3 days x 7 trials)

Trial	Moisture (g/100 g).		
	1st day	2nd day	3rd day
1	55.77	55.12	55.64
2	55.62	55.42	55.41
3	55.48	55.43	55.43
4	55.51	55.44	55.33
5	55.58	55.48	55.40
6	55.75	55.13	55.37
7	55.42	55.32	55.43
Repeatability relative standard deviation (%)	0.23		
Intermediate relative standard deviation (%) (intermediate precision)	0.31		

(2) Method of Protein content analysis

Protein content in two samples (sample C and D) were analyzed using AOAC 988.05 with slight modification that a 200 mL Kjeldahl flask was used, and the reagents used for hydrolysis were 1/2 scale. In addition, boiling stones [Sigma-Aldrich Japan LLC] were used instead of Alundum granules, and a few drops of 1% silicone defoamer were added as foaming was observed during distillation. Nitrogen / protein conversion factor 5.71 was used for calculation.

The results for sample C and D are shown in
Table 4 and Table 5, respectively.

Table 4 Analyses of protein content in sample C (3 days x 7 trials)

Trial	Protein (g/100 g).		
	1st day	2nd day	3rd day
1	15.92	16.00	15.83
2	16.03	15.95	15.68
3	15.61	15.92	15.83
4	15.83	15.83	15.77
5	15.76	15.78	15.82
6	15.81	15.83	15.66
7	15.83	15.75	15.74

Repeatability relative standard deviation (%)	0.64
Intermediate relative standard deviation (%) （intermediate precision）	0.68

Table 5 Analyses of protein content in sample D (3 days x 7 trials)

Trial	Protein (g/100 g).		
	1st day	2nd day	3rd day
1	13.37	13.24	13.56
2	12.88	13.34	13.47
3	13.42	13.66	13.63
4	13.16	13.55	13.38
5	13.50	13.82	13.75
6	13.85	13.62	13.44
7	13.03	13.75	13.55
Repeatability relative standard deviation (%)	1.74		
Intermediate relative standard deviation (%) （intermediate precision）	1.91		

(3) Method of Lipid content analysis

Lipid content in two samples (sample E and F) were analyzed using AOAC 963.15. Accurately weigh 4 g of natto was used as a test portion. Whatman grade 589/2, which is equivalent to S&S 589 filter paper described in the AOAC method, was used for the filter paper.

The results for sample E and F are shown in
Table 6 and Table 7, respectively.

Table 6 Analyses of lipid content in sample E (3 days x 7 trials)

Trial	Lipid (g/100 g).		
	1st day	2nd day	3rd day
1	10.03	9,99	9.97
2	10.04	10.00	9.97
3	10.70	10.02	10.01
4	9.86	10.00	9.97
5	9.99	10.02	10.02
6	9.38	10.04	10.09
7	9.94	9.79	10.06
Repeatability relative standard deviation (%)	1.52		
Intermediate relative standard deviation (%) （intermediate precision）	1.54		

Table 7 Analyses of lipid content in sample F (3 days x 7 trials)

Trial	Lipid (g/100 g).		
	1st day	2nd day	3rd day
1	7.02	6.70	7.27
2	7.12	7.26	7.25
3	7.16	7.15	7.23
4	7.19	7.26	7.25
5	7.08	7.09	7.35
6	7.18	7.24	7.29
7	7.26	7.30	7.17
Repeatability relative standard deviation (%)	1.79		
Intermediate relative standard deviation (%) (intermediate precision)	1.86		

Results

The summary of the study is shown in Table 8. According to AOAC OMA Appendix F: Guidelines for Standard Method Performance Requirements, the acceptable range for HorRat(r) is between 0.3 and 1.3. HorRat(r) in these trials are all sufficiently below the upper limit of generally acceptable range of HorRat(r).

Considering the above-mentioned fact, it is likely that RSD_R would meet the performance criteria stipulated in the Procedural Manual.

Table 8 Summary of single-laboratory validation study

Provision	Concentration	RSD_i [%]	RSD_r [%]	PRSD(R) [%][6]	HorRat(r)
Moisture	≧53%	0.13, 0.31	0.13, 0.23	1.37	0.095, 0.17
Protein	≧10%	0.68, 1.91	0.64, 1.74	2.83	0.23, 0.62
Lipid	≧5%	1.54, 1.86	1.52, 1.79	3.14	0.48, 0.57

[6] $PRSD(R)=C^{-0.5}$ ($C>0.138$) or $2C^{-0.15}$ ($C\leqq 0.138$), where C is concentration expressed as mass fraction.

Annex II

Validation studies for methods of analysis for *Cheonggukjang*

(submitted by Republic of Korea)

Background

To ensure applicability of these methods for *Cheonggukjang*, a single-laboratory validation study was conducted by Korea.

Validation study

Each analysis was conducted 7 times per day for 3 days.

1. *Cheonggukjang*

(1) Method of Moisture content analysis

Moisture content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 2 and Table 3, respectively.

Table 1 Analyses of moisture content in sample A (3 days x 7 trials)

Sample A Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	48.96	49.00	48.65	48.97	0.31	0.63
2	49.07	48.93	48.59			
3	49.86	49.28	49.00			
4	48.48	48.97	49.08			
5	49.20	49.13	48.89			
6	48.50	48.64	49.12			
7	49.14	48.81	49.01			
Each of Mean	49.03	48.97	48.91			
Mean r	48.97					
Each of SD	0.47	0.21	0.21			
SD r	0.21					
Each of RSD	0.96	0.42	0.43			
RSD r	0.43					

Table 2 Analyses of moisture content in sample B (3 days x 7 trials)

Sample B Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	51.71	51.58	51.70	51.60	0.10	0.20
2	51.62	51.77	51.56			
3	51.58	51.78	51.76			
4	51.53	51.54	51.56			
5	51.61	51.61	51.47			
6	51.41	51.56	51.56			

7	51.51	51.75	51.50			
Each of Mean	51.57	51.66	51.59			
Mean r	51.60					
Each of SD	0.10	0.11	0.10			
SD r	0.10					
Each of RSD	0.18	0.21	0.20			
RSD r	0.20					

(2) Method of Protein content analysis

Protein content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 3 and Table 4, respectively.

Table 3 Analyses of protein content in sample A (3 days x 7 trials)

Sample A Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	19.54	19.93	19.38	19.59	0.21	1.06
2	19.72	19.56	19.56			
3	19.48	19.89	19.70			
4	19.48	19.76	19.77			
5	19.50	19.44	19.39			
6	19.32	19.65	19.55			
7	20.03	19.31	19.34			
Each of Mean	19.58	19.65	19.53			
Mean r	19.59					
Each of SD	0.23	0.23	0.17			
SD r	0.23					
Each of RSD	1.18	1.17	0.85			
RSD r	1.17					

Table 4 Analyses of protein content in sample B (3 days x 7 trials)

Sample B Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	17.30	17.50	17.57	17.53	0.22	1.24
2	17.29	17.55	17.23			
3	17.41	17.95	17.69			
4	17.54	17.79	17.22			
5	17.50	17.81	17.40			
6	17.34	17.79	17.29			
7	17.53	17.85	17.52			
Each of Mean	17.41	17.75	17.42			
Mean r	17.53					

Each of SD	0.11	0.16	0.18
SD r	0.16		
Each of RSD	0.62	0.92	1.03
RSD r	0.92		

(3) Method of Lipid content analysis

Lipid content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 5 and Table 6, respectively.

Table 5 Analyses of lipid content in sample A (3 days x 7 trials)

Sample A Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	5.05	4.76	4.70	4.87	0.15	3.03
2	4.95	4.88	4.87			
3	4.66	4.62	4.98			
4	4.87	4.99	5.06			
5	4.91	4.74	4.66			
6	5.06	4.89	4.79			
7	4.72	4.96	5.11			
Each of Mean	4.89	4.83	4.88			
Mean r	4.87					
Each of SD	0.15	0.13	0.17			
SD r	0.15					
Each of RSD	3.12	2.73	3.55			
RSD r	3.12					

Table 6 Analyses of lipid content in sample B (3 days x 7 trials)

Sample B Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	4.33	4.19	4.01	4.15	0.11	2.59
2	3.99	4.10	4.19			
3	4.09	4.01	4.22			
4	4.01	4.24	3.99			
5	4.14	4.31	4.31			
6	4.23	4.18	4.22			
7	4.14	4.09	4.14			
Each of Mean	4.13	4.16	4.16			
Mean r	4.15					
Each of SD	0.12	0.10	0.12			
SD r	0.12					
Each of RSD	2.89	2.41	2.83			

RSD r	2.83

Results

The summary of the study is shown in Table 87. HorRat(r) values for moisture content were 0.144 and 0.303. HorRat(r) values for Protein content were 0.395 and 0.502. HorRat(r) values for Lipid content were 0.601 and 0.662.

Table 7 Summary of single-laboratory validation study

Provision	Concentration	RSD_i [%] A	RSD_i [%] B	RSD_r [%] A	RSD_r [%] B	Average of MEANi(s)	PRSD(R) [%][1]	HorRat r A	HorRat r B
Moisture	C(%) ≦ 51.77	0.63	0.20	0.43	0.20	50.29	1.41	0.303	0.144
Protein	C(%) ≧ 17.22	1.06	1.24	1.17	0.92	18.56	2.32	0.502	0.395
Lipid	C(%) ≧ 4.01	3.03	2.59	3.12	2.83	4.51	4.71	0.662	0.601

2. Cheonggukjang powder

(1) Method of Moisture content analysis

Moisture content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 2 and Table 3, respectively.

Table 8 Analyses of moisture content in sample A (3 days x 7 trials)

Sample A Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	6.81	6.85	7.09	6.88	0.11	1.63
2	6.82	6.82	7.00			
3	6.71	6.94	7.02			
4	6.73	6.86	7.00			
5	6.81	6.80	6.98			
6	6.74	6.89	7.06			
7	6.79	6.90	6.96			
Each of Mean	6.77	6.86	7.02			
Mean r	6.88					
Each of SD	0.04	0.05	0.05			
SD r	0.05					
Each of RSD	0.66	0.71	0.64			
RSD r	0.66					

Table 9 Analyses of moisture content in sample B (3 days x 7 trials)

Sample B Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	6.83	6.90	6.97	6.92	0.08	1.20

2	6.87	6.84	6.96			
3	6.87	6.81	7.09			
4	6.80	6.87	7.00			
5	6.88	6.96	6.99			
6	6.87	6.95	7.07			
7	6.87	6.86	7.03			
Each of Mean	6.86	6.88	7.02			
Mean r	6.92					
Each of SD	0.03	0.05	0.05			
SD r	0.05					
Each of RSD	0.42	0.79	0.70			
RSD r	0.70					

(2) Method of Protein content analysis

Protein content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 10 and Table 11, respectively.

Table 10 Analyses of protein content in sample A (3 days x 7 trials)

Sample A Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	39.28	40.16	38.29			
2	39.58	39.48	38.42			
3	39.32	39.50	38.79			
4	39.17	40.21	38.72	39.25	0.52	1.32
5	39.14	39.56	38.86			
6	39.47	40.15	38.97			
7	39.08	39.23	38.98			
Each of Mean	39.29	39.76	38.72			
Mean r	39.25					
Each of SD	0.18	0.41	0.27			
SD r	0.27					
Each of RSD	0.46	1.02	0.70			
RSD r	0.70					

Table 11 Analyses of protein content in sample B (3 days x 7 trials)

Sample B Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	39.80	39.88	38.35			
2	39.89	39.87	38.68	39.42	0.59	1.49
3	39.86	39.56	38.67			

4	39.77	39.90	38.39			
5	39.65	40.10	38.72			
6	39.57	39.90	38.89			
7	39.91	39.64	38.77			
Each of Mean	39.78	39.83	38.64			
Mean r	39.42					
Each of SD	0.13	0.18	0.20			
SD r	0.18					
Each of RSD	0.32	0.46	0.51			
RSD r	0.46					

(3) Method of Lipid content analysis

Lipid content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 12 and Table 13, respectively.

Table 12 Analyses of lipid content in sample A (3 days x 7 trials)

Sample A Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	25.43	25.41	25.52	25.43	0.14	0.54
2	25.57	25.23	25.42			
3	25.29	25.43	25.39			
4	25.50	25.32	25.49			
5	25.34	25.75	25.23			
6	25.60	25.47	25.42			
7	25.24	25.32	25.62			
Each of Mean	25.42	25.42	25.44			
Mean r	25.43					
Each of SD	0.14	0.17	0.12			
SD r	0.14					
Each of RSD	0.55	0.65	0.48			
RSD r	0.55					

Table 13 Analyses of lipid content in sample B (3 days x 7 trials)

Sample B Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	21.91	21.87	21.84	21.68	0.16	0.74
2	21.89	21.62	21.72			
3	21.53	21.76	21.62			
4	21.51	21.55	21.39			
5	21.92	21.47	21.88			

6	21.79	21.60	21.62			
7	21.62	21.73	21.55			
Each of Mean	21.74	21.65	21.66			
Mean r	21.68					
Each of SD	0.18	0.14	0.17			
SD r	0.17					
Each of RSD	0.85	0.63	0.78			
RSD r	0.78					

Results

The summary of the study is shown in Table 814. HorRat(r) values for moisture content were 0.174 and 0.185. HorRat(r) values for Protein content were 0.287 and 0.437. HorRat(r) values for Lipid content were 0.265 and 0.381.

Table 14 Summary of single-laboratory validation study

Provision	Concentration	RSD_i [%] A	RSD_i [%] B	RSD_r [%] A	RSD_r [%] B	Average of MEANi(s)	PRSD(R) [%][1]	HorRat r A	HorRat r B
Moisture	C(%) ≦ 7.09	1.63	1.20	0.66	0.70	6.90	3.81	0.174	0.185
Protein	C(%) ≧ 38.29	1.32	1.49	0.70	0.46	39.34	1.59	0.437	0.287
Lipid	C(%) ≧ 21.39	0.54	0.74	0.55	0.78	23.56	2.06	0.265	0.381

3. *Cheonggukjang* spherical pellet

(1) Method of Moisture content analysis

Moisture content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 2 and Table 3, respectively.

Table 15 Analyses of moisture content in sample A (3 days x 7 trials)

Sample A Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	4.05	4.12	4.10			
2	3.99	4.07	4.16			
3	4.05	4.12	4.11			
4	4.01	4.15	4.16	4.09	0.06	1.39
5	4.00	4.11	4.08			
6	4.06	4.17	4.10			
7	3.99	4.10	4.13			
Each of Mean	4.02	4.12	4.12			
Mean r	4.09					
Each of SD	0.03	0.03	0.03			
SD r	0.03					
Each of RSD	0.79	0.75	0.75			
RSD r	0.75					

Table 16 Analyses of moisture content in sample B (3 days x 7 trials)

Sample B Trial	Moisture (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	3.94	4.04	4.12	4.02	0.08	1.94
2	3.96	4.00	4.12			
3	3.87	4.02	4.10			
4	3.92	4.05	4.04			
5	3.94	4.02	4.12			
6	3.92	4.09	4.08			
7	3.94	4.03	4.11			
Each of Mean	3.93	4.03	4.10			
Mean r	4.02					
Each of SD	0.03	0.03	0.03			
SD r	0.03					
Each of RSD	0.75	0.75	0.71			
RSD r	0.75					

(2) Method of Protein content analysis

Protein content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 17 and Table 18, respectively.

Table 17 Analyses of protein content in sample A (3 days x 7 trials)

Sample A Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	42.38	42.45	42.22	42.51	0.24	0.57
2	42.36	42.55	42.30			
3	42.48	42.43	42.52			
4	42.49	42.72	42.37			
5	43.41	42.51	42.37			
6	42.54	42.76	42.52			
7	42.59	42.35	42.50			
Each of Mean	42.61	42.54	42.40			
Mean r	42.51					
Each of SD	0.36	0.15	0.12			
SD r	0.15					
Each of RSD	0.85	0.35	0.28			
RSD r	0.35					

Table 18 Analyses of protein content in sample B (3 days x 7 trials)

Sample B Trial	Protein (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	39.43	38.96	38.95	39.16	0.27	0.68
2	39.20	39.22	39.07			
3	39.33	39.25	39.11			
4	38.62	38.96	39.98			
5	39.11	39.14	39.02			
6	39.33	39.39	38.90			
7	39.11	39.30	39.07			
Each of Mean	39.16	39.17	39.16			
Mean r	39.16					
Each of SD	0.27	0.17	0.37			
SD r	0.27					
Each of RSD	0.68	0.42	0.95			
RSD r	0.68					

(3) Method of Lipid content analysis

Lipid content in two samples (sample A and B) were analyzed 7 times per day for 3 days.

The results for sample A and B are shown in Table 19 and Table 20, respectively.

Table 19 Analyses of lipid content in sample A (3 days x 7 trials)

Sample A Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	18.50	18.45	18.52	18.56	0.13	0.69
2	18.51	18.60	18.62			
3	18.65	18.78	18.59			
4	18.57	18.36	18.36			
5	18.43	18.67	18.67			
6	18.33	18.74	18.42			
7	18.63	18.62	18.66			
Each of Mean	18.52	18.60	18.55			
Mean r	18.56					
Each of SD	0.11	0.15	0.12			
SD r	0.12					
Each of RSD	0.61	0.80	0.66			
RSD r	0.66					

Table 20 Analyses of lipid content in sample B (3 days x 7 trials)

Sample B Trial	Lipid (g/100 g).			Intermediate		
	1st day	2nd day	3rd day	MEANi	SDi	RSDi
1	23.76	23.61	23.52	23.57	0.17	0.71
2	23.24	23.56	23.72			
3	23.64	23.26	23.56			
4	23.52	23.67	23.52			
5	23.80	23.72	23.31			
6	23.50	23.82	23.80			
7	23.56	23.50	23.46			
Each of Mean	23.57	23.59	23.55			
Mean r	23.57					
Each of SD	0.19	0.18	0.16			
SD r	0.18					
Each of RSD	0.80	0.75	0.69			
RSD r	0.75					

Results

The summary of the study is shown in Table 8. HorRat(r) values for moisture content were 0.150 and 0.151. HorRat(r) values for Protein content were 0.226 and 0.436. HorRat(r) values for Lipid content were 0.301 and 0.346.

Table 21 Summary of single-laboratory validation study

Provision	Concentration	RSD_i [%] A	RSD_i [%] B	RSD_r [%] A	RSD_r [%] B	Average of MEANi(s)	PRSD(R) [%][1]	HorRat r A	HorRat r B
Moisture	C(%) ≦ 4.17	1.39	1.94	0.75	0.75	4.05	4.97	0.151	0.150
Protein	C(%) ≧ 38.62	0.57	0.68	0.35	0.68	40.84	1.56	0.226	0.436
Lipid	C(%) ≧ 18.33	0.69	0.71	0.66	0.75	21.06	2.18	0.301	0.346

Appendix II

LIST OF PARTICIPANTS

No.	Country
Member countries	
1	China
2	Indonesia
3	Japan (Chair)
4	Malaysia
5	Republic of Korea
6	Thailand
7	Vietnam
Observer countries	
8	France
9	Nigeria
10	Uganda