CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 2 **CRD 4**

Original language only

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECCIAL DIETARY USES

Thirty-eighth Session

Hamburg, Germany, 5 – 9 December 2016

MATTERS REFERRED TO THE COMMITTEE BY THE CODEX ALIMENTARIUS COMMISSION AND/OR OTHER SUBSIDIARY BODIES

Comments of European Union, Kenya, African Union, IDF and ISDI

EUROPEAN UNION

Part B. MATTERS ARISING FROM SUBSIDIARY BODIES AS RELATED TO THE WORK OF CCNFSDU

Committee on Methods of Analysis and Sampling (CCMAS37)

Mixed competence

Member States vote

Protein conversion factors

The European Union and its Member States have taken note of the conclusions of CCMAS and would support the establishment of a FAO/WHO expert panel to review available literature to assess the scientific basis for protein conversion factors and to possibly update the report of the joint FAO/WHO/UNU expert consultation *Protein and Amino Acid Requirements in Human Nutrition* (2002).

Examination of "ELISA G12" as a potential method for inclusion in the Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CODEX STAN 118-1979)

Last year, CCNFSDU decided to ask CCMAS to provide further clarification on the question of the methods for detection of gluten. In particular, CCNFSDU asked: "*Taking into account that the thresholds in CODEX STAN 118-1979 were established on the basis of the results given by the ELISA R5 Method, can CCMAS confirm that the results of the two methods (R5 and G12) are fully comparable for all products covered by the standard, in particular:*

- *products manufactured from ingredients naturally free of gluten (e.g. buckwheat, millet, amaranth, quinoa, etc.);*
- *products manufactured from gluten-containing ingredients (e.g. partially hydrolysed wheat protein, wheat starch, malt extract, glucose syrups, etc.);*
- *products based on oats; and*
- *liquid matrices*".

Since CCMAS explained in its reply that the two methods (R5 and G12) are not comparable, that comparability data for the two methods were not available, and mixed matrices are not included in the scope of either of the methods obtained during their validation, the European Union and its Member States are of the view that ELISA G12 cannot for the moment be included in the Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CODEX STAN 118-1979).

Committee on Food Additives (CCFA48)

European Union competence

European Union vote

Gellan gum (INS 418)

In considering the use of food additives in infant formula, formula for special medical purposes for infants and follow-up formula the approach discussed and proposed by JECFA in 1971 implemented by the Codex Alimentarius Commission and endorsed by the 43rd Session of the Codex Committee on Food Additives that

"*baby foods should be prepared without food additives whenever possible. Where the use of a food additive becomes necessary in baby foods, great caution should be exercised regarding both the choice of additive and its level of use*" shall apply.

Infant formula, formula for special medical purposes for infants and follow-up formula are on the EU market and are produced without the use of gellan gum. Therefore, in the EU's view gellan gum is not necessary and not technologically justified for the aforementioned foods.

Flavourings

The EU supports revising the text pertaining to flavourings in the standards referred to in para 24 of CX/NFSDU 16/38/2 in order to ensure consistency the *Guidelines for the Use of Flavourings* (CAC/GL 66-2008) provided such revision keeps the restrictions for the use of flavourings as currently listed in the mentioned standards (e.g. for Codex STAN 73-1981 only vanilla extract at GMP, ethyl vanillin at 7 mg and vanillin at 7 mg is permitted).

KENYA

Paragraph 7: Consistency of the Risk Analysis Texts across Relevant Committees

Issue: CAC39 endorsed the recommendations of CCGP8 that CCNFSDU should revise the text on nutritional risk analysis and consider how to include JEMNU as a primary source of scientific advice.

Comment: We support the revision of Nutritional Risk Analysis Principles and Guidelines for Application to the Work of the Committee on Nutrition and Foods to make Joint FAO/WHO Expert Meeting on Nutrition (JEMNU) as proposed below:

'Para 33. Consistent with ~~their~~ its important role in providing scientific advice to the Codex Alimentarius Commission and its subsidiary bodies, ~~FAO and WHO~~ JEMNU ~~are~~ is acknowledged as the primary source of nutritional risk assessment advice to Codex Alimentarius. ~~This acknowledgement however, does not preclude the possible consideration of recommendations arising from other internationally recognised expert bodies, as approved by the Commission.~~

Justification: This inclusion will ensure that CCNFSDU has a single authoritative reference for technical advice as opposed to the current situation where several scientific bodies opinion which at times differ are recognized by CCNFSDU.

Paragraph 18: Examination of "ELISA G12" as a potential method for inclusion in the Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CODEX STAN 118-1979)

Issue: CCMAS37 agreed that the two methods (R5 and G12) are not comparable, that comparability data for the two methods were not available, and mixed matrices are not included in the scope of either of the methods obtained during their validation.

Comment: There is need to establish an expert committee meeting to review the two methods in details.

Justification: The basis of response of CCMAS is not clear given that they have indicated that there was lack of comparability data. There is need to for an expert meeting to review the two methods in details including where possible calling for data so as to make a final decision. The expert should also recommend of the two methods the most acceptable/reliable to use.

Paragraph 22: Gellan gum (INS 418)

Issue: CCFA48 requested CCNFSDU to confirm the technological need of gellan gum (INS 418) in infant formula, formula for special medical purposes for infants, and follow-up formula.

Comment: There is no technological need for use of Gellan gum as requested by Codex Committee on Food Additives (CCFA).

Justification: Effort should be to reduce to the great extent possible the use of food additives in products made for infants. In addition, the food category has number food additives options to be used a stabilizers/thickeners. CCFA should advice on the safety of use Gellan gun especially within the under 12 weeks old.

AFRICAN UNION

Paragraph 7: Consistency of the Risk Analysis Texts across Relevant Committees

Issue: CAC39 endorsed the recommendations of CCGP28 that CCNFSDU should revise the text on nutritional risk analysis and consider how to include JEMNU as a primary source of scientific advice.

Comment: The AU proposes the revision of the Nutritional Risk Analysis Principles and Guidelines for Application to the Work of the Committee on Nutrition and Foods for Special Dietary Uses which was

adopted in 2009 by amending paragraph 33 of section IV of the procedural manual by replacing 'WHO and FAO' with Joint FAO/WHO Expert Meeting on Nutrition (JEMNU). Further, the last sentence of paragraph 33 should be deleted as indicated below

'Para 33. Consistent with ~~their~~ its important role in providing scientific advice to the Codex Alimentarius Commission and its subsidiary bodies, ~~FAO and WHO~~ JEMNU ~~are~~ is acknowledged as the primary source of nutritional risk assessment advice to Codex Alimentarius. ~~This acknowledgement however, does not preclude the possible consideration of recommendations arising from other internationally recognised expert bodies, as approved by the Commission.~~

Rationale: AU appreciates the need of a primary source of advice to the committee as opposed to the current situation where the committee is relying on recommendation of Recognized Authoritative Scientific Bodies (RASBs) to make decision on various matter. JEMNU being an expert committee will be able to interrogate any scientific information and provide to the committee a considered opinion as in the case with other Codex committees.

Paragraph 16: 37th Session of the Committee on Methods of Analysis and Sampling (CCMAS37) Protein Conversion factors

Issue: CCMAS37 agreed that it was not in a position to reply to the questions posed by CCNFSDU37 as the determination of conversion factors was in the remit of CCNFSDU.

Comment: The AU recommends that FAO/WHO consider convening an expert meeting to determine the appropriate conversion factor for soya.

Rationale: Taking note of the reply from Codex Committee on Method of Analysis and Sampling (CCMAS), indicating that it was the mandate of CCNFSDU and given that this committee had decided to seek assistance, it will be important for an expert meeting to be convened to address the issue conclusively and advice CCNFSDU on the right conversion factor

Paragraph 18: Examination of "ELISA G12" as a potential method for inclusion in the Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CODEX STAN 118-1979)

Issue: CCMAS37 agreed that the two methods (R5 and G12) are not comparable, that comparability data for the two methods were not available, and mixed matrices are not included in the scope of either of the methods obtained during their validation.

Comment: The AU recommends that CCNFSDU requests CCMAS to consider reviewing the two methods with a view of clarification of their reply.

Rationale: It is not clear from the reply on the basis of the decision to conclude that the two methods are not comparable given that the reply indicates that there is no comparability data for the methods.

Paragraph 22: Gellan gum (INS 418)

Issue: CCFA48 requested CCNFSDU to confirm the technological need of gellan gum (INS 418) in infant formula, formula for special medical purposes for infants, and follow-up formula.

Comment: There is no technological need for use of Gellan gum as requested by Codex Committee on Food Additives (CCFA).

Rationale: There is need for safety data on exposure to the additive especially for infants under 12 weeks. Effort should be made to reduce to the extent possible the use of food additives in products made for infants.

IDF – International Dairy Federation

Protein Conversion factors

Introduction

At the 37th session of CCMAS, IDF submitted the results of a review of the scientific literature on nitrogen conversion factors for soy protein[1].

The main conclusions were:

- Scientific publications based on experimental and/or theoretical analysis of NCFs consistently demonstrate that the use of an NCF of 6.25 for soy protein products is incorrect and scientifically flawed and overestimates the soy protein content by 8–9%.

[1] MAS/37 CRD/5

- For soy products in general, the scientific literature reports NCFs in the range 5.6–5.8. The only value quoted higher than this range (6.30, for soy flour) was obtained through erroneous exclusion of nitrogen content from the amides contained in asparagine and glutamine.
- The NCF for soy protein *isolates* (range 5.63–5.85; mean 5.73) is not substantially different from that for other soy products.
- On the basis of limited data, the NCF for soy hydrolysates (range 5.56–5.59; mean 5.58) appears to be similar to that for other soy products.
- Allowing for wide variation in the ratio of 11S to 7S proteins from different soy cultivars, the calculated NCF for soy-based infant formulas ranges from 5.69 to 5.79 (mean 5.74). This mean is not significantly different from the value of 5.71 stated in the Codex Standard for Infant Formula.

In this CRD, IDF wishes to provide supplementary data regarding the calculation of NCFs for soy protein. This data shows that on the basis of amino acid composition calculations for soy proteins the NCFs reported in earlier scientific publications, assuming an amide:acid ratio of 50:50, is entirely consistent with current knowledge of the amino acid composition of soy proteins.

Amino acid sequences of soy proteins were obtained from UniProt[2]. Table 1 shows the amide:acid ratio based on sequence and the calculated NCF values. Full data are shown in Appendices 1-5.

Table 1 Amide:acid ratios based on UniProt amino acid sequences, and NCF values calculated from these sequences

Protein	**Amide:acid ratio based on sequence**	**Calculated NCF**
2S albumin (small and large chains)	32:68	5.94
7S β-conglycinin α	44:56	5.65
7S β-conglycinin α'	45:55	5.58
7S β-conglycinin β	53:47	5.66
11S glycinin (A3+B4 subunits)	54:46	5.49

The data in Table 1[3] clearly show that the average 50:50 amide:acid ratio for soy protein put forward in the majority of publications is entirely consistent with information calculated from currently known amino acid sequences.

It therefore follows that the values for NCFs reported for soy protein in MAS/37 CRD/5 are scientifically accurate estimates and justify the use of NCF=5.71 for soy protein.

NCF values of about 6.3 for soy protein can *only* be obtained by *excluding* asparagine and glutamine. In other words, reported values of about 6.3 can only be obtained by using an amide:acid ratio of 0:100 (see Appendix 6). As the amide:acid ratio in soy protein cannot be 0:100, any claims supporting a NCF value of about 6.3 have been based on erroneous calculations. This can be corroborated by, for example, performing calculations based on:

- The study of Zheng *et al.*[4], which shows that for β-conglycinin a value of NCF=6.31 can only be obtained when using an amide:acid ratio of 0:100. However, if the ratio is changed to 50:50 (as per UniProt data) then the NCF value is 5.57 (Appendix 7).
- Historical data for isolated soy protein as reported by AOCS (CX/NFSDU 16/38/6-Add.1, Appendix 1, page 66). NCF values reported (range 6.30-6.37) have evidently been calculated using an amide:acid ratio of 0:100 (Appendix 6 and Appendix 8). Using the same data and an amide:acid ratio of 50:50, the NCF value is 5.69 (Appendix 8), in accordance with scientific literature and UniProt data.

Conclusions

[2] Dataset UniProtKB/Swiss-Prot; www.uniprot.org. This is a constantly evolving up-to-date database. For all proteins cited in Appendices 1-5 of this CRD, the most recent modifications to the UniProt data occurred in 2016.
[3] Note that the fraction of 2S albumins is small and has a proportionally small effect on the average ratio.
[4] Zheng H-G, Yang X-Q, Ahmad I, Min W, Zhu J-H and Yuan D-B (2009) Soybean b-conglycinin constituent subunits: Isolation, solubility and amino acid composition. Food Res Intl 42:998-1003.

Calculations yielding NCFs for soy protein of about 6.3 can only be obtained when excluding asparagine and glutamine. Such an approach is evidently erroneous. The various studies in the scientific literature using an average amide:acid ratio of 50:50 for soy protein are employing best scientific practice, are entirely consistent with information calculated from currently known amino acid sequences, and justify the use of NCF=5.71 for soy protein.

APPENDIX 1

2S ALBUMIN

http://www.uniprot.org/uniprot/P19594

Small chain	SKWQHQQDSCRKQLQGVNLTPCEKHIMEKIQGRGDDDDDDDDD
Large chain	EGKDEDEEEEGHMQKCCTEMSELRSPKCQCKALQKIMENQSEELEEKQKKKMEKELINLATMC RFGPMIQCDLSSDD

Asn:Asp	17:83
Gln:Glu	41:59
Amide:Acid	32:68

AA[5]		MW AA[6]	MW res[7]	%N in res[8]	Small[9]	Large[10]	Total[11]	Res total[12]	Res N[13]
A	Ala	89.1	71.1	19.69	0	2	2	142.2	28.00
R	Arg	174.2	156.2	35.85	2	2	4	624.8	224.00
D	Asp	133.1	115.1	12.16	10	5	15	1726.5	209.98
N	Asn	132.1	114.1	24.54	1	2	3	342.3	83.99
C	Cys	121.2	103.2	13.57	2	6	8	825.6	112.00
E	Glu	147.1	129.1	10.84	2	15	17	2194.7	237.94
Q	Gln	146.1	128.1	21.85	6	6	12	1537.2	335.87
G	Gly	75.1	57.1	24.53	3	3	6	342.6	84.04
H	His	155.2	137.2	30.62	2	1	3	411.6	126.04
I	Ile	131.2	113.2	12.37	2	3	5	566	70.01
L	Leu	131.2	113.2	12.37	2	6	8	905.6	112.02
K	Lys	146.2	128.2	21.84	4	10	14	1794.8	392.03
M	Met	149.2	131.2	10.67	1	6	7	918.4	97.99
F	Phe	165.2	147.2	9.51	0	1	1	147.2	14.00
P	Pro	115.1	97.1	14.41	1	2	3	291.3	41.99
S	Ser	105.1	87.1	16.08	2	5	7	609.7	98.01
T	Thr	119.1	101.1	13.84	1	2	3	303.3	41.99
W	Trp	204.2	186.2	15.04	1	0	1	186.2	28.00

[5] One letter and three letter amino acid codes.
[6] Molecular weight of amino acid (g AA/mol).
[7] Molecular weight anhydrous amino acid residue (g anhydrous AA/mol equivalent).
[8] Percentage nitrogen in anhydrous amino acid residue (g nitrogen/g anhydrous AA).
[9] Number of amino acid residues in small chain.
[10] Number of amino acid residues in large chain.
[11] Sum of number of amino acid residues in small and large chains.
[12] Total amino acid residues calculated as “MW res” x “Total” (g anhydrous AA/mol protein).
[13] Total nitrogen contribution of the amino acid calculated as “Res total” x “%N in res” (g nitrogen/mol protein).

AA[5]		MW AA[6]	MW res[7]	%N in res[8]	Small[9]	Large[10]	Total[11]	Res total[12]	Res N[13]
Y	Tyr	181.2	163.2	8.58	0	0	0	0	0.00
V	Val	117.2	99.2	14.12	1	0	1	99.2	14.01
	TOTAL				43	77	120	13969	2352
								NCF :	5.94

APPENDIX 2

7S B-CONGLYCININ α

http://www.uniprot.org/uniprot/P13916

β-conglycinin α	VEKEECEEGEIPRPRPRPQHPEREPQQPGEKEEDEDEQPRPIPFPRPQPRQEEEHEQ REEQEWPRKEEKRGEKGSEEEDEDEDEEQDERQFPFPRPPHQKEERNEEEDEDEEQ QRESEESEDSELRRHKNKNPFLFGSNRFETLFKNQYGRIRVLQRFNQRSPQLQNLRDY RILEFNSKPNTLLLPNHADADYLIVILNGTAILSLVNNDDRDSYRLQSGDALRVPSGTTYY VVNPDNNENLRLITLAIPVNKPGRFESFFLSSTEAQQSYLQGFSRNILEASYDTKFEEINK VLFSREEGQQQGEQRLQESVIVEISKEQIRALSKRAKSSSRKTISSEDKPFNLRSRDPIY SNKLGKFFEITPEKNPQLRDLDIFLSIVDMNEGALLLPHFNSKAIVILVINEGDANIELVGLK EQQQEQQQEEQPLEVRKYRAELSEQDIFVIPAGYPVVVNATSNLNFFAIGINAENNQRN FLAGSQDNVISQIPSQVQELAFPGSAQAVEKLLKNQRESYFVDAQPKKKEEGNKGRKG PLSSILRAFY

Asn:Asp	58:42
Gln:Glu	37:63
Amide:Acid	44:56

AA		MW AA	MW res	%N in res	# Res	Res Total	Res N
A	Ala	89.1	71.1	19.69	23	1635.3	322.00
R	Arg	174.2	156.2	35.85	43	6716.6	2408.00
D	Asp	133.1	115.1	12.16	27	3107.7	377.97
N	Asn	132.1	114.1	24.54	37	4221.7	1035.82
C	Cys	121.2	103.2	13.57	1	103.2	14.00
E	Glu	147.1	129.1	10.84	77	9940.7	1077.75
Q	Gln	146.1	128.1	21.85	45	5764.5	1259.51
G	Gly	75.1	57.1	24.53	24	1370.4	336.18
H	His	155.2	137.2	30.62	6	823.2	252.07
I	Ile	131.2	113.2	12.37	30	3396	420.07
L	Leu	131.2	113.2	12.37	45	5094	630.11
K	Lys	146.2	128.2	21.84	31	3974.2	868.07
M	Met	149.2	131.2	10.67	1	131.2	14.00
F	Phe	165.2	147.2	9.51	27	3974.4	378.03
P	Pro	115.1	97.1	14.41	38	3689.8	531.84

AA		MW AA	MW res	%N in res	# Res	Res Total	Res N
S	Ser	105.1	87.1	16.08	39	3396.9	546.06
T	Thr	119.1	101.1	13.84	11	1112.1	153.97
W	Trp	204.2	186.2	15.04	1	186.2	28.00
Y	Tyr	181.2	163.2	8.58	13	2121.6	182.01
V	Val	117.2	99.2	14.12	24	2380.8	336.17
	TOTAL				543	63141	11172
						NCF=5.65	

APPENDIX 3

7S B-CONGLYCININ α'

http://www.uniprot.org/uniprot/P11827

β-conglycinin α'	VEEEEECEEGQIPRPRPQHPERERQQHGEKEEDEGEQPRPFPFPRPRQPHQEEEHEQKEEHEWH RKEEKHGGKGSEEEQDEREHPRPHQPHQKEEEKHEWQHKQEKHQGKESEEEEEDQDEDEEQDK ESQESEGSESQREPRRHKNKNPFHFNSKRFQTLFKNQYGHVRVLQRFNKRSQQLQNLRDYRILEF NSKPNTLLLPHHADADYLIVILNGTAILTLVNNDDRDSYNLQSGDALRVPAGTTFYVVNPDNDENLRM IAGTTFYVVNPDNDENLRMITLAIPVNKPGRFESFFLSSTQAQQSYLQGFSKNILEASYDTKFEEINKV LFGREEGQQQGEERLQESVIVEISKKQIRELSKHAKSSSRKTISSEDKPFNLGSRDPIYSNKLGKLFEI TQRNPQLRDLDVFLSVVDMNEGALFLPHFNSKAIVVLVINEGEANIELVGIKEQQQRQQQEEQPLEV RKYRAELSEQDIFVIPAGYPVMVNATSDLNFFAFGINAENNQRNFLAGSKDNVISQIPSQVQELAFPR SAKDIENLIKSQSESYFVDAQPQQKEEGNKGRKGPLSSILRAFY

Asn:Asp	57:43
Gln:Glu	40:60
Amide:Acid	45:55

AA		MW AA	MW res	%N in res	#res	Res Total	Res N
A	Ala	89.1	71.1	19.69	23	1635.3	322.00
R	Arg	174.2	156.2	35.85	38	5935.6	2128.00
D	Asp	133.1	115.1	12.16	28	3222.8	391.97
N	Asn	132.1	114.1	24.54	37	4221.7	1035.82
C	Cys	121.2	103.2	13.57	1	103.2	14.00
E	Glu	147.1	129.1	10.84	79	10198.9	1105.74
Q	Gln	146.1	128.1	21.85	52	6661.2	1455.43
G	Gly	75.1	57.1	24.53	29	1655.9	406.21
H	His	155.2	137.2	30.62	20	2744	840.24
I	Ile	131.2	113.2	12.37	28	3169.6	392.07
L	Leu	131.2	113.2	12.37	41	4641.2	574.10
K	Lys	146.2	128.2	21.84	38	4871.6	1064.08
M	Met	149.2	131.2	10.67	4	524.8	56.00

AA		MW AA	MW res	%N in res	#res	Res Total	Res N
F	Phe	165.2	147.2	9.51	29	4268.8	406.03
P	Pro	115.1	97.1	14.41	33	3204.3	461.86
S	Ser	105.1	87.1	16.08	40	3484	560.06
T	Thr	119.1	101.1	13.84	14	1415.4	195.96
W	Trp	204.2	186.2	15.04	2	372.4	56.00
Y	Tyr	181.2	163.2	8.58	13	2121.6	182.01
V	Val	117.2	99.2	14.12	28	2777.6	392.20
	TOTAL				577	67230	12040
						NCF=5.58	

APPENDIX 4

7S B-CONGLYCININ β

http://www.uniprot.org/uniprot/P25974

β-conglycinin β	LKVREDENNPFYFRSSNSFQTLFENQNVRIRLLQRFNKRSPQLENLRDYRIVQFQSKPNTILLPHHA DADFLLFVLSGRAILTLVNNDDRDSYNLHPGDAQRIPAGTTYYLVNPHDHQNLKIIKLAIPVNKPGRYD DFFLSSTQAQQSYLQGFSHNILETSFHSEFEEINRVLFGEEEEQRQQEGVIVELSKEQIRQLSRRAKS SSRKTISSEDEPFNLRSRNPIYSNNFGKFFEITPEKNPQLRDLDIFLSSVDINEGALLLPHFNSKAIVILV INEGDANIELVGIKEQQQKQKQEEEPLEVQRYRAELSEDDVFVIPAAYPFVVNATSNLNFLAFGINAE NNQRNFLAGEKDNVVRQIERQVQELAFPGSAQDVERLLKKQRESYFVDAQPQQKEEGSKGRKGPF PSILGALY

Asn:Asp	61:39
Gln:Glu	47:53
Amide:Acid	53:47

AA		MW AA	MW res	%N in res	#res	Res Total	Res N
A	Ala	89.1	71.1	19.69	22	1564.2	308.00
R	Arg	174.2	156.2	35.85	29	4529.8	1624.00
D	Asp	133.1	115.1	12.16	21	2417.1	293.97
N	Asn	132.1	114.1	24.54	33	3765.3	923.84
C	Cys	121.2	103.2	13.57	0	0	0.00
E	Glu	147.1	129.1	10.84	37	4776.7	517.88
Q	Gln	146.1	128.1	21.85	33	4227.3	923.64
G	Gly	75.1	57.1	24.53	18	1027.8	252.13
H	His	155.2	137.2	30.62	8	1097.6	336.10
I	Ile	131.2	113.2	12.37	26	2943.2	364.06
L	Leu	131.2	113.2	12.37	42	4754.4	588.10
K	Lys	146.2	128.2	21.84	21	2692.2	588.05

AA		MW AA	MW res	%N in res	#res	Res Total	Res N
M	Met	149.2	131.2	10.67	0	0	0.00
F	Phe	165.2	147.2	9.51	28	4121.6	392.03
P	Pro	115.1	97.1	14.41	21	2039.1	293.91
S	Ser	105.1	87.1	16.08	31	2700.1	434.05
T	Thr	119.1	101.1	13.84	10	1011	139.97
W	Trp	204.2	186.2	15.04	0	0	0.00
Y	Tyr	181.2	163.2	8.58	12	1958.4	168.01
V	Val	117.2	99.2	14.12	24	2380.8	336.17
	TOTAL				416	48007	8484
						NCF=5.66	

APPENDIX 5

11S GLYCININ

http://www.uniprot.org/uniprot/P04347

A3 subunit	ITSSKFNECQLNNLNALEPDHRVESEGGLIETWNSQHPELQCAGVTVSKRTLNRNGSHLPSYLPYPQ MIIVVQGKGAIGFAFPGCPETFEKPQQQSSRRGSRSQQQLQDSHQKIRHFNEGDVLVIPLGVPYWTY NTGDEPVVAISPLDTSNFNNQLDQNPRVFYLAGNPDIEHPETMQQQQQQKSHGGRKQGQHRQQEE EGGSVLSGFSKHFLAQSFNTNEDTAEKLRSPDDERKQIVTVEGGLSVISPKWQEQEDEDEDEDEEYG RTPSYPPRRPSHGKHEDDEDEDEEEDQPRPDHPPQRPSRPEQQEPRGRGCQTRN
B4 subunit	GVEENICTMKLHENIARPSRADFYNPKAGRISTLNSLTLPALRQFGLSAQYVVLYRNGIYSPDWNLNA NSVTMTRGKGRVRVVNCQGNAVFDGELRRGQLLVVPQNPAVAEQGGEQGLEYVVFKTHHNAVSSY IKDVFRVIPSEVLSNSYNLGQSQVRQLKYQGNSGPLVNP

Asn:Asp	58:42
Gln:Glu	52:48
Amide:Acid	54:46

AA		MW AA	MW res	%N in res	A3	B4	Total	Res. Total	Res N
A	Ala	89.1	71.1	19.69	8	10	18	1279.8	252.00
R	Arg	174.2	156.2	35.85	21	12	33	5154.6	1848.00
D	Asp	133.1	115.1	12.16	20	4	24	2762.4	335.97
N	Asn	132.1	114.1	24.54	17	16	33	3765.3	923.84
C	Cys	121.2	103.2	13.57	4	2	6	619.2	84.00
E	Glu	147.1	129.1	10.84	34	8	42	5422.2	587.86
Q	Gln	146.1	128.1	21.85	34	11	45	5764.5	1259.51
G	Gly	75.1	57.1	24.53	25	15	40	2284	560.29
H	His	155.2	137.2	30.62	12	3	15	2058	630.18

AA		MW AA	MW res	%N in res	A3	B4	Total	Res. Total	Res N
I	Ile	131.2	113.2	12.37	11	6	17	1924.4	238.04
L	Leu	131.2	113.2	12.37	18	16	34	3848.8	476.08
K	Lys	146.2	128.2	21.84	12	6	18	2307.6	504.04
M	Met	149.2	131.2	10.67	2	2	4	524.8	56.00
F	Phe	165.2	147.2	9.51	10	5	15	2208	210.01
P	Pro	115.1	97.1	14.41	28	9	37	3592.7	517.84
S	Ser	105.1	87.1	16.08	25	13	38	3309.8	532.06
T	Thr	119.1	101.1	13.84	14	6	20	2022	279.94
W	Trp	204.2	186.2	15.04	3	1	4	744.8	112.00
Y	Tyr	181.2	163.2	8.58	7	8	15	2448	210.01
V	Val	117.2	99.2	14.12	15	19	34	3372.8	476.24
	TOTAL				320	172	492	55414	10094
								NCF=5.49	

APPENDIX 6 CALCULATION OF NCF FOR SOY PROTEIN FROM HISTORICAL DATA *EXCLUDES* ASPARAGINE AND GLUTAMINE[14]

AA[15]	2004	2004	2004	2004	2005	2005	2005	2005	2006	2006	2006	2006	2006	2006	2007	2007	2007	2007	avg	%N	AN[16]
Ala	3.56	3.32	3.36	3.41	3.45	3.4	3.35	3.37	3.4	3.24	3.38	3.39	3.36	3.4	3.34	3.29	3.34	3.37	3.37	19.6	0.66
Arg	6.86	6.8	6.71	6.76	6.75	6.84	6.84	6.74	6.58	6.79	6.72	6.7	6.8	6.72	6.72	6.68	6.57	6.62	6.73	35.8	2.41
Asp	10.2	9.74	9.7	9.84	10.1	10.2	10.1	9.77	9.66	9.97	10.0	10.0	9.82	10.0	9.77	9.62	9.66	9.77	9.91	12.1	1.20
Cyst	1.03	1.14	1.06	1.05	1.08	1.05	1.08	1.03	1.08	1.11	1.06	0.99	1.02	1.05	1.03	1.06	1.02	1.05	1.06	13.5	0.14
GlutA	18.2	18.1	17.9	18.1	18.4	18.7	18.6	18.2	16.2	18.6	18.1	17.9	19.2	18.3	16.7	16.4	16.4	16.4	17.8	10.8	1.93
Glyc	3.17	3.08	3.04	3.09	3.13	3.13	3.08	3.08	3.08	3.05	3.06	3.08	3.09	3.12	3.06	3.02	3.05	3.04	3.08	24.5	0.76
Hist	2.11	2.09	2.04	2.05	2.06	2.08	2.02	2.07	2.04	2.02	2.04	2.02	2.12	2.09	2.11	2.09	2.07	2.08	2.07	30.6	0.63
Isolu	3.98	3.73	3.74	3.79	3.87	3.82	3.87	3.74	3.74	3.76	3.89	3.94	3.78	3.93	3.87	3.83	3.81	3.91	3.83	12.3	0.47
Leu	7.2	6.77	6.83	6.93	6.94	6.89	6.92	6.75	6.85	6.68	6.97	7.08	6.9	6.88	6.77	6.7	6.75	6.84	6.87	12.3	0.85
Lys	5.54	5.51	5.37	5.45	5.43	5.51	5.36	5.44	5.35	5.38	5.33	6.37	5.44	5.44	5.5	5.37	5.39	5.36	5.47	21.8	1.20
Meth	1.12	1.27	1.23	1.19	1.15	1.11	1.13	1.13	1.16	1.16	1.1	1.09	1.14	1.14	1.11	1.14	1.15	1.14	1.15	10.6	0.12
Phenyl	4.82	4.5	4.46	4.6	4.69	4.66	4.69	4.47	4.45	4.45	4.73	4.81	4.57	4.55	4.51	4.42	4.4	4.53	4.57	9.51	0.43
Pro	4.36	4.77	4.48	4.4	4.54	4.49	4.5	4.48	4.6	4.58	4.73	4.55	4.64	4.79	4.5	4.5	4.33	4.45	4.54	14.4	0.65
Ser	4.34	4.16	4.11	4.23	4.26	4.26	4.22	4.1	4.19	4.16	4.2	4.22	4.18	4.09	4.08	4.03	4.01	4.04	4.16	16.0	0.67
Thr	3.19	3.06	3.05	3.1	3.17	3.15	3.11	3.04	3.11	2.95	3.06	3.05	3.04	3.12	3.01	3.01	3.02	3.06	3.07	13.8	0.43
Trypto	1.09	1.11	1.14	1.09	1.06	1.07	1.02	1.07	1.09	1.06	1.07	1.05	1.05	1.12	0.99	1.09	1.12	1.11	1.08	15.0	0.16
Tyr	3.57	3.41	3.35	3.49	3.46	3.41	3.48	3.4	3.46	3.36	3.45	3.51	3.44	3.45	3.43	3.33	3.37	3.37	3.43	8.58	0.29
Val	4.16	4.15	4.13	4.06	4.15	4.13	4.15	4.09	3.99	3.88	4.07	4.08	4.1	4.4	4.17	4.2	4.18	4.24	4.13	14.1	0.58
Sum[17]	88.6	86.7	85.7	86.6	87.7	87.9	87.6	85.9	84.0	86.2	87.0	87.9	87.7	87.7	84.7	83.8	83.6	84.4	86.3		
TotalN	13.9	13.6	13.5	13.6	13.8	13.8	13.7	13.5	13.3	13.5	13.6	13.8	13.7	13.8	13.4	13.3	13.2	13.3	13.6		13.6
NCF	6.35	6.33	6.34	6.35	6.36	6.35	6.37	6.34	6.31	6.35	6.37	6.34	6.36	6.36	6.31	6.3	6.31	6.32	6.34		

[14] Data obtained from AOCS (CX/NFSDU 16/38/6-Add.1, Appendix 1, page 66); units: g anhydrous AA/100g sample.
[15] Amino acid. Note that Asn and Gln have been excluded.
[16] Average nitrogen contribution calculated as the average amino acid value times %N in residue, divided by 100 (g nitrogen/100g sample).
[17] Total protein content, calculated as the sum of the amino acid values (g protein/100g sample).
[18] Total nitrogen content, calculated as the sum of the nitrogen contributions of each amino acid, as exemplified in the last column (g nitrogen/100g sample).

APPENDIX 7

CALCULATIONS OF NCF VALUES BASED ON ZHENG *ET AL*[19]

	0% GlX/AsX are amide			50% GlX/AsX are amide		
AA	**Average**[20]	**%N in res**	**Res N**[21]	**Average**	**%N in res**	**Res N**
Ala	3.99	19.69	0.79	3.99	19.69	0.79
Arg	8.28	35.85	2.97	8.28	35.85	2.97
Asp	12.04	12.16	1.46	6.02	12.16	0.73
Asn		24.54	0.00	6.02	24.54	1.48
Cyst	0.03	13.57	0.00	0.03	13.57	0.00
GlutA	24.53	10.84	2.66	12.27	10.84	1.33
Gln		21.85	0.00	12.27	21.85	2.68
Glyc	3.47	24.53	0.85	3.47	24.53	0.85
Hist	2.84	30.62	0.87	2.84	30.62	0.87
Isolu	4.54	12.37	0.56	4.54	12.37	0.56
Leu	7.5	12.37	0.93	7.50	12.37	0.93
Lys	6.09	21.84	1.33	6.09	21.84	1.33
Meth	0.39	10.67	0.04	0.39	10.67	0.04
PhenylA	5.38	9.51	0.51	5.38	9.51	0.51
Pro	4.74	14.41	0.68	4.74	14.41	0.68
Ser	5.38	16.08	0.86	5.38	16.08	0.86
Thr	3.25	13.84	0.45	3.25	13.84	0.45
Trypto	0.00	15.04	0.00	0.00	15.04	0.00
Tyr	3.45	8.58	0.30	3.45	8.58	0.30
Val	4.09	14.12	0.58	4.09	14.12	0.58
Total	99.99		15.85	99.99		17.94
	NCF=6.31			NCF= 5.57		

APPENDIX 8

COMPARISON OF CALCULATIONS USING HISTORICAL DATA[22]: NCFs OBTAINED WHEN USING AMIDE:ACID RATIO OF 0:100 OR 50:50, RESPECTIVELY

	0% GlX/AsX amide			50% GlX/AsX amide		
AA	**Average**[23]	**%N in res**	**Res N**[24]	**Average**	**%N in res**	**Res N**
Ala	3.37	19.69	0.664	3.37	19.69	0.664
Arg	6.73	35.85	2.414	6.73	35.85	2.414
Asp	9.90	12.16	1.205	4.95	12.16	0.602

[19] Zheng H-G, Yang X-Q, Ahmad I, Min W, Zhu J-H and Yuan D-B (2009) Soybean b-conglycinin consitutent subunits: Isolation, solubility and amino acid composition. Food Res Intl 42:998-1003.
[20] g anhydrous AA/100 g protein.
[21] g nitrogen/100 g protein.
[22] From AOCS (CX/NFSDU 16/38/6-Add.1, Appendix 1, page 66).
[23] g anhydrous AA/100g sample.
[24] g nitrogen/100 g sample.

Asn	0	24.54	0	4.95	24.54	1.215
Cyst	1.06	13.57	0.143	1.06	13.57	0.143
GlutA	17.84	10.84	1.934	8.92	10.84	0.967
Gln	0	21.85	0	8.92	21.85	1.918
Glyc	3.08	24.53	0.766	3.08	24.53	0.766
Hist	2.07	30.62	0.633	2.07	30.62	0.633
Isolu	3.83	12.37	0.474	3.83	12.37	0.474
Leu	6.87	12.37	0.850	6.87	12.37	0.850
Lys	5.47	21.84	1.196	5.47	21.84	1.196
Meth	1.15	10.67	0.123	1.15	10.67	0.123
PhenylA	4.57	9.51	0.435	4.57	9.51	0.435
Pro	4.54	14.41	0.654	4.54	14.41	0.654
Ser	4.16	16.08	0.669	4.16	16.08	0.669
Thr	3.07	13.84	0.425	3.07	13.84	0.425
Trypto	1.08	15.04	0.162	1.08	15.04	0.162
Tyr	3.43	8.58	0.294	3.43	8.58	0.294
Val	4.13	14.12	0.583	4.13	14.12	0.583
Total	86.36		13.61	86.36		15.19
	NCF=6.34			NCF=5.69		

ISDI – International Special Dietary Foods Industries

22. Technological need for Gellan gum (INS 418)

CCFA48 requested CCNFSDU to confirm the technological need for gellan gum (INS 418) in infant formula, formula for special medical purposes for infants, and follow-up formula.

a) At CCFA48 ISDI requested a JECFA priority evaluation of gellan gum (INS 418) as a thickener up to 0.005 g/100 mL as consumed, in hydrolyzed protein and/or amino acid based formula only, formula for special medical purposes (FSMP) for infants, food category 13.1.3. CCFA then requested that CCNFSDU first confirm the technological need for gellan gum.

b) This confirms that, consistent with Codex guidance (CAC/GL 36-1989), gellan gum (INS 418) is a thickener with a technological purpose to enable increasing the viscosity of the food. ISDI has provided a complete description of the technological justification for gellan gum as an Annex to this document.

c) Based on confirmation of the technological justification of gellan gum (INS 418) ISDI requests that the 38th CCNFSDU provide a reference to CCFA recommending the addition of gellan gum to the JECFA priority list for safety evaluation for use in formulas for infants.

24. Flavourings

CCFA48 recommended to CCNFSDU to consider revising the text pertaining to flavourings in the following standards to ensure consistency with the *Guidelines for the Use of Flavourings*:

Standard for Canned Baby Foods (CODEX STAN 73-1981)

Standard for Processed Cereal-Based Foods for Infants and Yong Children (CODEX STAN 74-1981)

Standard for Follow-up Formula (CODEX STAN 156-1987)

a) ISDI supports the recommendation to address the issue of inconsistent terminologies related to flavourings between Codex *Guidelines for the Use of Flavourings* and the Codex texts that are within the mandate of CCNFSDU. (CX/FA 15/47/20 Discussion Paper)

b) ISDI agrees that flavourings are food additives and as such their use should be addressed in the Food Additives section of Codex commodity standards, and should contain a reference to the *Guidelines*. (Recommendation III of CX/FA 15/47/20 Discussion Paper)

c) ISDI supports revising the text to use the term “Flavouring” in place of “Flavor” in the following standards:

i. *Standard for Canned Baby Foods* (CODEX STAN 73-1981) (section 4.5)

ii. *Standard for Processed Cereal-Based Foods for Infants and Yong Children* (CODEX STAN 74-1981) (section 3.9)

iii. *Standard for Follow-up Formula* (CODEX STAN 156-1987) (section 4.5)

Summary of ISDI Position Agenda Item #2

Gellan gum

- Request CCNFSDU to confirm the technological justification for gellan gum (INS 418), and provide a reference to CCFA recommending the addition of gellan gum as a thickener up to 0.005 g/100 mL to the JECFA priority list for safety assessment for use in formulas for infants.

Flavourings

- Support revision of text to use the term “Flavouring” in place of “Flavour” in the 3 standards identified, and make the following general reference to the *Guidelines* in the Flavouring sections:

> “The flavourings used in products covered by this standard shall comply with the *Guidelines for the Use of Flavourings (CAC/GL 66-2008)”*

Annex I

Technological justification for the use of gellan gum

GELLAN GUM (INS 418)

Introduction

ISDI responded to CL 2015/11-FA with a proposal for a JECFA priority evaluation of gellan gum (INS 418) as a thickener up to 0.005 g/100 mL in hydrolyzed protein and/or amino acid based formula only, formula for special medical purposes (FSMP) for infants, food category 13.1.3. ISDI requests that the CCNFSDU Committee provide a reference to CCFA recommending the addition of gellan gum to the JECFA priority list for safety evaluation based on technological justification.

In this conference room document (CRD), ISDI provides the 38th CCNFSDU with a summary of the technological justification for the use of gellan gum.

Technological justification

Gellan gum acts as a thickener/stabilizer in ready-to-feed infant formula, or concentrated liquid products through formation of a fluid gel that can aid with the sedimentation of dense components such as insoluble calcium and phosphorus salts. This provides a secondary benefit of thickening the solution, slowing the upward migration of fat, which is less dense. Gellan gum stabilizes the emulsion of protein, fat and water created in the infant formula manufacturing process, minimizing phase separation during storage, display and feeding. Without an ingredient added for stabilization, infant formula would be more likely to produce insoluble sediments or creaming (separation of fat). This technical effect is particularly important to ensure infant formula is homogenous and delivers the appropriate level of all essential nutrients. Use of product that is not properly stabilized will result in suboptimal delivery of nutrients to an infant, and long-term use could result in nutrient deficiency. Infant formula products can uniquely benefit from these multifunctional properties of gellan gum.

Advantages

Gellan gum is cold or hot water-soluble, which allows for advantageous flexibility of addition for manufacturing applications. It also has good thermal and acid stability. Full hydration of the gum occurs during thermal processing temperatures used in infant formula ensuring desired effectivity of the stabilizer. The elasticity of the gel obtained from gellan gum is adjustable based on presence of ions, pH, or temperature. Therefore, gellan gum can be adapted to improve the physical stability of a variety of nutritionally complete, low viscosity formulas. Another benefit of gellan gum is that it does not influence the efficacy of the other components, particularly the vitamins and minerals in the formulation. Thus, gellan gum is compatible with formulation processing, allowing the minimum undesirable impact on the ingredients and during subsequent storage.

SPIFAN METHODS provided by ISDI

SPIFAN METHODS provided by International Special Dietary Foods Industries (ISDI)

B. MATTERS ARISING FROM SUBSIDIARY BODIES AS RELATED TO THE WORK OF CCNFSDU

MATTERS FOR ACTION

37th Session of the Codex Committee on Methods of Analysis and Sampling (CCMAS37)

Methods of Analysis and Sampling

Executive Summary

This document presents information from the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) in response to feedback provided by the Codex Committee on Methods of Analysis and Sampling (CCMAS) regarding infant formula methods of analysis.

Recommendations to CCNFSDU

The International Special Dietary Foods Industries (ISDI), on behalf of the SPIFAN community, recommends CCNFSDU take the following actions:

1. Chromium/Selenium/Molybdenum – Refer AOAC 2011.19 | ISO 20649 | IDF 235 to CCMAS for endorsement as Type II.
2. Vitamin B12 – Designate AOAC 986.23 as Type IV.
3. Fatty Acids – Designate AOAC 996.06 as Type III.
4. Myo-inositol – Endorse AOAC 2011.18 | ISO 20637 as Type II.
5. Vitamin E – Endorse AOAC 2012.10 | ISO 20633 as Type II and reclass EN 12822 as Type III.
6. Other general considerations – Adopt a proposed explanatory text for the conversion from amounts per gram or kilogram to amounts per 100 kcal or kJ.

Background on Method Types (from Codex Procedural Manual[25])

(a) Defining Methods (Type I)

Definition: A method which determines a value that can only be arrived at in terms of the method per se and serves by definition as the only method for establishing the accepted value of the item measured.

(b) Reference Methods (Type II)

Definition: A Type II method is the one designated Reference Method where Type I methods do not apply. It should be selected from Type III methods (as defined below). ***It should be recommended for use in cases of dispute*** and or for calibration purposes.

(c) Alternative Approved Methods (Type III)

Definition: A Type III Method is one which meets the criteria required by the Committee on Method of Analysis and Sampling for methods that may be used for control, inspection or regulatory purposes.

(d) Tentative Method (Type IV)

Definition: A Type IV Method is a method which has been used traditionally or else has been recently introduced but for which the criteria required for acceptance by the Committee on Methods of Analysis and Sampling have not yet been determined.

Information Regarding Specific Methods of Analysis

Chromium, Selenium and Molybdenum – AOAC 2011.19 | ISO 20649 | IDF 235

During its 37th Session, CCMAS considered AOAC 2011.19 | ISO 20649 | IDF 235, which measures chromium, selenium and molybdenum, as a possible Type II method. Although the method was extensively validated specifically for infant formula, is more precise and necessary for use to ensure the nutritional safety of infant formula products, CCMAS agreed to ask CCNFSDU for further guidance relating to the criteria and, pending the outcome, to propose to CAC to adopt AOAC 2011.19 | ISO 20649 | IDF 235 as a type III method.

[25] Codex Alimentarius Commission Procedural Manual, 25th Edition.

The CCMAS report indicated the method was not endorsed as Type II due to concerns that the method requires expensive instrumentation and some countries may not have the capacity to run the method. It should be noted a Type II method is not required to be used except in the case of resolving a dispute that cannot otherwise be settled, and in all other cases any approved Codex method may be used. The SPIFAN community supports the desire of countries to use all approved Codex methods for routine nutrient analysis. However, there is a strong need to have one Type II method for each nutrient or group of nutrients in infant formula that will be used as the referee method in the case of a dispute that cannot otherwise be settled. Note, the ICP-MS instrumentation used in AOAC 2011.19 | ISO 20649 | IDF 235 is the same as that used in AOAC 2012.15 | ISO 20647 | IDF 234, a method for total iodine which was adopted by the Codex Alimentarius Commission as Type II in July 2016.

CCMAS also noted that the method provision in CODEX STAN 72-1981 indicated that none of the methods for these analytes, including current Codex methods and the proposed SPIFAN method, would meet the criteria, specifically the minimum limit (ML). CCMAS asked CCNFSDU to review the numeric values for the method criteria and provide feedback to CCMAS on the correct values and how to proceed.

In response to this feedback from CCMAS, the method author for AOAC 2011.19 provided additional validation data on the qualification limit of the method as well as reproducibility data on placebos. AOAC INTERNATIONAL published this information in *JAOAC,* which summarizes the additional work and demonstrates that the method, without modifications, operates at or above the quantitation limit and demonstrates acceptable reproducibility at the minimum levels set through CODEX STAN 72-1981.[26]

While CCMAS suggested that CCNFSDU consider a criteria approach regarding methods for these analytes, the regulatory community needs a Type II method for the purpose of dispute resolution. A criteria approach does not meet these needs. Further, AOAC 2011.19 | ISO 20649 | IDF 235 is the only method that meets the Codex method criteria, which supports it becoming a Type II method.

It is recommended that CCNFSDU confirm to CCMAS that a dispute resolution method is needed for these analytes and, as AOAC 2011.19 | ISO 20649 | IDF 235 is the only method that meets the method criteria, this method should be endorsed as a Type II method. This recommendation is in line with that made by CCNFSDU during its 37th Session.

Vitamin B12 – AOAC 2011.10 | ISO 20634

During its 37th Session, CCMAS endorsed this method as Type II and agreed to request CCNFSDU to clarify whether the existing method in CODEX STAN 234-1999 is still fit for purpose and, if so, whether it would become Type III.

AOAC 986.23 is based on a non-specific determination known to respond to substances other than cobalamin, resulting in a potential bias with AOAC 2011.10 | ISO 20634, which is based on HPLC-UV.[27] In addition, AOAC 986.23 is validated using only milk based infant formula, which is not representative of all current infant formula matrices on the market. Furthermore, the precision estimates were based on a collaborative study that does not meet current requirements (e.g., the joint International Union of Pure and Applied Chemistry, International Organization for Standardization, and AOAC harmonized protocol[28]). Hence, it does not meet Codex requirements for Type II or Type III methods.

AOAC 2011.10 | ISO 20634 was validated on a broad range of different matrices and has improved precision and accuracy compared to AOAC 986.23, where poor repeatability and a high number of failed results has been observed due to poor growth of the organism and/or contamination. Although AOAC 986.23 shows high sensitivity, enabling the detection of low concentrations other food components can cause interference with the assay. It is also expensive to support in the absence of a minimum level of use.

For the above reasons, it is recommended that AOAC 986.23 be reclassified as Type IV. The SPIFAN community believes the process to reclassify AOAC 986.23 as a Type IV method should happen independently of CAC adoption of AOAC 2011.10 | ISO 20634 as a Type II method.

Table 1. Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981) – METHODS OF ANALYSIS

Commodity	Provision	Method	Principle	Type

[26] Thompson and Pacquette. Characterization of AOAC Final Action Official Method 2011.19 and AOAC First Action Official Method 2015.06 Performance at Analyte Levels Corresponding to CODEX STAN 72-1981 Minimum Levels. JAOAC INTERNTIONAL. Published online November 2016. http://ingentaconnect.com/content/aoac/jaoac/pre-prints/content-160325

[27] Campos-Giménez et al. JAOAC INTERNATIONAL Vol 91, No4, 2008: 786. (See page 793, paragraph 'Comparison with MBA').

[28] Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Appendix D.

Infant Formula	Vitamin B12	AOAC 2011.10 **|** ISO 20634	HPLC	II
		AOAC 986.23 Total B12 as cyanocobalamin	**Turbidimetric**	**~~II~~ IV**

Fatty Acids (including trans fatty acids) – AOAC 2012.13 | ISO 16958 | IDF 231

During its 37th Session, CCMAS recommended to change the wording in the provision for the method description, endorsed the method as Type II and further recommended the existing method be changed to Type III.

ISDI notes the Matters Referred document does not indicate any Matters for Action related to fatty acids. However, as CCMAS endorsed AOAC 2012.13 | ISO 16958 | IDF 231 as a Type II and recommended AOAC 996.06 be changed to a Type III, it is necessary for CCNFSDU to confirm this recommendation. It is therefore recommended that CCNFSDU confirm AOAC 996.06 should become Type III.

Table 1. Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981) – METHODS OF ANALYSIS

Commodity	Provision	Method	Principle	Type
Infant Formula	~~Total Fatty Acid Profile Fatty acids~~ Fatty acids (including trans fatty acids)	AOAC 2012.13 **|** ISO 16958 | IDF 231	Gas Chromatography	II
	Fatty Acids (including trans fatty acid)	**AOAC 996.06**	**Gas chromatography**	**~~II~~ III**
		AOCS Ce ~~1h 05~~ 1i-07	**Gas chromatography**	**III**

Myo-Inositol – AOAC 2011.18 | ISO 20637

Introduction

The proposed method (ISO 20637/AOAC 2011.18, Determination of Myo-Inositol) was considered by the Codex Committee on Methods of Analysis and Sampling (CCMAS) for inclusion as a Type II method in the Recommended Methods of Analysis and Sampling (CODEX STAN 234-1999), Infant Formula (IF) Section A, during its 37th Session. Section A applies to IF in liquid or powdered form intended for use, where necessary, as a substitute for human milk in meeting the normal nutritional requirements of infants (CODEX STAN 72-1981). CCMAS raised the following point:

"The Committee agreed to request CCNSFDU to confirm that the AOAC 2011.18 and ISO 20637 determine the forms to be measured according to CODEX STAN 72-1981 for myo-inositol. The AOAC 2011.18 and ISO 20637 determine free and bound myo-inositol as phosphatidylinositol, but it is unclear if this is the definition (inclusion of free and bound) in CODEX STAN 72-1981. Provided that the definition and the scope of the methods harmonize, CCMAS recommended endorsement of AOAC 2011.18 and ISO 20637 as Type II. (It does not need to come back for re-endorsement by CCMAS.)"

Supporting Information

CAC GL-10, "Advisory Lists of Nutrient Compounds for use In Foods for Special Dietary Uses Intended for Infants and Young Children," lists myo-inositol (meso-inositol) as the nutrient source of inositol in IF Section A, but does not define myo-inositol.

During the 30th CCNFSDU Session, the Electronic Working Group on Methods of Analysis for Infant Formula and Formulas for Special Medical Purposes Intended for Infants noted CODEX STAN 72-1981 is "reasonably specific for myo-inositol" and commented that the "current standard implies that both free and/or phospholipid-bound myo-inositol may be included and requires clarification."[29]

In 2010, the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) myo-inositol Working Group, comprised of experts from all over the world, defined myo-inositol as free myo-inositol (CAS

[29] Joint FAO/WHO Food Standards Programme. Codex Committee on Nutrition and Foods for Special Dietary Uses, 30th Session, Cape Town, Africa. Report of the Electronic Working Group on Methods of Analysis for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981). September 2008. ftp://ftp.fao.org/codex/Meetings/CCNFSDU/ccnfsdu30/nf3002ae.pdf

87-89-8) and phosphatidylinositol, but excluding methyl ethers, glycosides, phosphorylated forms, and phytate.[30] In 2011 the AOAC SPIFAN approved this definition of myo-inositol.

Currently Codex has not endorsed any methods for the determination of myo-inositol in the Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. In 2015, AOAC 2011.18 | ISO 20637, Determination of myo-Inositol was submitted to the Codex Committee on Nutrition and Foods for Special Dietary Uses for consideration during its 37th Session and referred to CCMAS for technical review, typing and possible inclusion as a Type II method.

ISO 20637/AOAC 2011.18 is specific for myo-inositol. With this method, free myo-inositol and myo-inositol bound as phosphatidylinositol are determined separately and the data added together to determine total myo-inositol as defined by AOAC SMPR 2011.007.

Conclusion

The AOAC SPIFAN definition of myo-inositol is interpreted to be consistent with the inositol forms the CCNFSDU Electronic Working Group on Methods of Analysis for Infant Formula and Formulas for Special Medical Purposes Intended for Infants previously concluded should be included in the definition of myo-inositol (i.e., free and/or phospholipid-bound myo-inositol). Determination of myo-inositol by AOAC 2011.19 | ISO 20637 meets the scope of the AOAC SPIFAN definition and what we understand are the CCNFSDU criteria for myo-inositol.

Table 1. Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981) – METHODS OF ANALYSIS

Commodity	Provision	Method	Principle	Type
Infant Formula	Myo-inositol	AOAC 2011.18 | ISO 20637	LC-pulsed amperometry	II

Vitamin E – AOAC 2012.10 | ISO 20633

During its 37th Session, CCMAS agreed to request CCNSFDU to confirm that the scope of AOAC 2012.10 | ISO 20633 is consistent with the provision for the isomers of vitamin E in CODEX STAN 72-1981. It was noted the method does not discriminate both d and dl-α-tocopherol and CODEX STAN 72-1981 refers only to d-α-tocopherol, but sources of vitamin E in CAC/GL 10-1979 include d-α-tocopherol, dl-α-tocopherol, d-α-tocopheryl acetate, dl-α-tocopheryl acetate, d-α-tocopheryl acid succinate, d-α-tocopheryl acid succinate, and dl-α-tocopheryl polyethylene glycol 1000 succinate. Provided that the provision and the scope of the method harmonize, CCMAS recommended endorsement of AOAC 2012.10 | ISO 20633 as Type II and noted this method then does not need to go back to CCMAS for re-endorsement.

Following are SPIFAN responses to CCMAS questions.

Vitamin E listed in the Advisory List of Nutrient Compounds for use in foods for special and dietary uses intended for infants and young children (CAC/GL10-1979) include several forms, while the Footnote of CODEX STAN 72-1981 refers to d-α-tocopherol only. Is the scope of the proposed method in line with the provisions for the isomers of vitamin E?

As per CAC GL-10, 'Advisory Lists of Nutrient Compounds for Use in Foods for Special Dietary Uses Intended for Infants and Young Children', d-α-tocopherol, dl-α-tocopherol, d-α-tocopheryl acetate, and dl-α-tocopheryl acetate are allowed for IF Section A.

Vitamin E succinates (d-α-tocopheryl acid succinate, dl-α-tocopheryl acid succinate and dl-α-tocopheryl polyethylene glycol 1000 succinate) are only allowed for IF Section B. Infant Formula Section B applies to Formula for Special Medical Purposes Intended for Infants in liquid or powdered form intended for use, where necessary, as a substitute for human milk or infant formula in meeting the special nutritional requirements arising from the disorder, disease or medical condition for whose dietary management the product has been formulated (CODEX STAN 72-1981).

Infant Formula Section B formulas are excluded from the scope of the proposed method.

Current Codex Type II (EN 12822) and proposed method AOAC 2012.10 | ISO 20633 do not distinguish between d- and dl-α-tocopherol.

Vitamin E consists of a group of eight closely related chemical substances: four tocopherols and four tocotrienols, which differ greatly in terms of their vitamin E activity.

[30] AOAC SMPR 2011.007 APPENDIX 1: AOAC SMPR 2011.007: JOURNAL OF AOAC INTERNATIONAL VOL. 95, NO. 2, 2012.

The naturally-occurring d- (or RRR) α-tocopherol is the most biologically active form and vitamin E activity is traditionally expressed in terms of equivalents of this isomer (mg α-tocopherol equivalents or α-TE). The most common supplemented form in infant formula is synthetic dl- (or all-rac) α-tocopherol acetate which consist of a mixture of active and inactive stereoisomers.

The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) has not yet decided on which vitamin E forms have biological activity, according to the report of the 39th Session of the Codex Alimentarius Commission (REP 16/NFSDU). However, the World Health Organization reported in 2016 that α-tocopherol is the only isomer with vitamin E activity. [31]

AOAC 2012.10 | ISO 20633 determines total vitamin E in infant formula. However, the method chromatographically separates the naturally-occurring d- (or RRR) α-tocopherol from the synthetic form, dl- (or all-rac) a-tocopherol acetate. The proposed method is the most recent scientific method of analysis for these vitamin forms in infant formula. Technology to distinguish between d and dl forms is available but not implemented yet in a validated method for infant formula.

Advantage of proposed method AOAC 2012.10 | ISO 20633 compared with current Type II: EN 12822.

The basis for reporting "vitamin E content", according to CODEX STAN 72-1981, is *"α-tocopherol equivalents" (TE).* This means that compounds to be included as "Vitamin E" need to be corrected for their biological activity. If a product contains natural tocopherol sources and synthetic tocopherol esters, it is necessary to apply the correct factors to end up with an accurate result. A method determining total α-tocopherol after saponification (e.g. EN 12822) only gives the correct value if the exact forms of the ingredients are known. For fortification purposes, manufacturers usually fortify with dl-α-tocopherol acetate (or dl-α-tocopherol). α-tocopherol in infant formula originates from natural oils used in its manufacture. The assumption is made that this form is d-α-tocopherol. If the fortified form is synthetic dl-α-tocopherol acetate, a factor of 1.49 is used to convert to the natural form d-α-tocopherol. If the fortified form is synthetic dl-α-tocopherol, a factor of 1.35 is used to convert to the natural d-α-tocopherol form. The proposed method AOAC 2012.10 | ISO 20633 will give a better estimation of the actual vitamin E content in the formula, expressed as α-tocopherol equivalents, (α- TE).

The Footnote in CODEX STAN-72-1981 refers to d-α-tocopherol.

This footnote refers to the expression of total vitamin E in infant formula as α-tocopherol equivalents (TE). Since 1 mg of vitamin E = 1 mg of d-α-tocopherol = 1 mg α-tocopherol equivalents (TE), other allowed vitamin E sources (CAC/GL10-1979) need to be corrected for their biological activity with the appropriate factor as described above.

Conclusion

The forms of vitamin E as stipulated in CODEX STAN 72-1981 and CAC/GL 10-1979, including d-α-tocopherol, dl-α-tocopherol, d-α-tocopheryl acetate, and dl-α-tocopheryl acetate are all included in the estimation of vitamin E and can be determined using the proposed method, AOAC 2012.10 | ISO 20633. This method will give a more accurate estimation of the actual vitamin E content in infant formula, expressed as α-tocopherol equivalents, (α- TE), compared to the current Type II method EN 12822.

Table 1. Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981) – METHODS OF ANALYSIS

Commodity	Provision	Method	Principle	Type
Infant Formula	Total Vitamin E (dl-α-Tocopherol and dl-α-Tocopherol Acetate)	AOAC 2012. 10 | ISO 20633	HPLC	II
	Vitamin E	**AOAC 992.03 Measures all rac-vitamin E (both natural + supplemental ester forms) aggregated and quantified as α-congeners**	**HPLC**	**III**
		EN 12822 (Measures Vitamin E (both natural +		

[31] World Health Organization and Food and Agriculture Organization of the United Nations. Guidelines on food fortification with micronutrients. Edited by Lindsay Allen, Bruno de Benoist, Omar Dary and Richard Hurrell. http://www.who.int/nutrition/publications/guide_food_fortification_micronutrients.pdf

		supplemental ester forms) aggregated and quantified as individual tocopherol congeners (α, β, γ, δ).	**HPLC**	**~~II~~ III**

Expression of Results Using Proposed Methods of Analysis

Results obtained by using the proposed methods of analysis for nutrients in infant formula are calculated and expressed in amounts per 100 g powder, or per 100 g Ready to Feed (RTF) product. RTF samples can be from liquid origin. When RTF is reconstituted from powders, 25 grams of powdered infant formula is to be mixed with 200 grams of water.
In CODEX STAN 72-1981, the essential composition is expressed in amounts per 100 available kilocalories, and amounts per 100 available kilojoules.

By using the amount of kcal and kJ per 100 g powder, or RTF product, on the product label of the sample analyzed, the nutrient concentrations can be calculated and expressed in amounts per 100 kcal or kJ as follows:

$$w = \frac{v}{y}\ x\ 100\ x\ f$$

w = nutrient concentration in mg/100 kcal or kJ
v = nutrient concentration in mg/100 g
y = amount of kcal or kJ per 100 g powder or RTF as indicated on sample package
f = dilution factor:
Example 1: In case of analysis of powders and of liquid Infant formula, f=1
Example 2: In case of reconstituted powders (25 g powder with 200 g of water), f=9.

Recommendations to CCNFSDU

ISDI, on behalf of the SPIFAN community, recommends CCNFSDU take the following actions:

1. Refer AOAC 2011.19 | ISO 20649 | IDF 235 to CCMAS for endorsement as Type II.
2. Designate AOAC 986.23 as Type IV.
3. Designate AOAC 996.06 as Type III.
4. Endorse AOAC 2011.18 | ISO 20637 as Type II.
5. Endorse AOAC 2012.10 | ISO 20633 as Type II and reclass EN 12822 as Type III.
6. Adopt a proposed explanatory text for the conversion from amounts per gram or kilogram to amounts per 100 kcal or kJ.