Agenda Item 2 **MAS/37 CRD/5**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS SAMPLING

Thirty-seventhth Session
Budapest, Hungary, 22 – 26 February 2016

MATTERS REFFERED BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER CODEX COMMITEES

(information provided by IDF)

Summary

The protein content of foods is commonly calculated by multiplying the analytically measured nitrogen content of a sample of food by a so-called nitrogen conversion factor (NCF). The use of scientifically appropriate NCFs for different foodstuffs is important for nutritional, sustainability and regulatory purposes.

The 37th session of the Codex Committee on Methods of Analysis and Sampling (CCMAS) (Budapest, 22-26 February 2016) has been asked to assess the accuracy and appropriateness of an NCF value of 5.71 for:

- determination of protein content in soybean products in general;
- soy protein used in formula for infants and young children, taking into account the amino acid profile of the isolate.

About a decade ago, Codex considered the NCFs for both soy products and milk products. IDF contributed to the Codex deliberations at that time by sharing its findings from a review of the literature [1], which concluded that there was no scientific justification to change the NCF for soy from 5.71 to 6.25. In light of the current discussions within Codex, IDF has provided in this submission an updated review of the scientific literature for soy proteins.

Key conclusions

- The overwhelming consensus of scientific studies is that specific NCFs for specific foods should be used.
- 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 is incorrect and scientifically flawed. Hence, the use of NCF=6.25 for soy protein products overestimates the protein content by 8–9%.
- 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.
- For *soy isolates*, data reported in the literature indicate that the NCFs for these products (range 5.63–5.85; mean 5.73) are not substantially different from those for other soy products.
- For *soy hydrolysates*, the limited data reported in the literature indicate that the NCFs for these products (range 5.56–5.59; mean 5.58) appear to be similar to those for other soy products.
- Allowing for wide variation in the ratio of 11S to 7S proteins from different soy cultivars, the calculated NCFs for soy-based infant formulas range from 5.69 to 5.79 (mean 5.74). The mean value is very close to the value of 5.71 stated in the Codex Standard for Infant Formula [2] as applicable to soy-based infant formula.

IDF also wish to draw the attention of CCMAS delegates to the paper by Maubois & Lorient (2016) relating to nitrogen conversion factors for dairy proteins and soy proteins in infant foods. This is an open access paper that may be freely distributed, and is available as part of IDF comments and through the following link: http://link.springer.com/article/10.1007/s13594-015-0271-0.

TABLE OF CONTENTS

1. INTRODUCTION

Protein is a principal nutrient in the human diet and its content in foods is commonly measured by determining the amount of nitrogen and multiplying that by a specific factor: the nitrogen conversion factor[1] (NCF). The NCF can be determined either by calculation from known protein composition and amino acid sequences, or by measuring the nitrogen content of a highly purified protein.

Hence, to formulate foods and to verify compliance with labelling requirements and other specifications, manufacturers and official control laboratories need to use scientifically justified NCFs that are ratified by international food standardization bodies such as the Codex Alimentarius Commission (CAC).

At its meeting in July 2015, the CAC decided to ask the Codex Committee on Methods of Analysis and Sampling (CCMAS) to "assess the appropriateness of the use of the conversion factor of 5.71 to determine protein content in soybean products in general" [3].

In view of this decision, the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) agreed to request CCMAS to "provide advice on the accuracy and appropriateness of 5.71 as the nitrogen factor for soy protein used in formula for infants and young children and to take into account the amino acid profile of the isolate" [4].

About a decade ago, Codex considered the appropriateness of NCFs for soy products and milk products. IDF contributed to the discussion at that time by reviewing the scientific literature [1]. In light of the current discussions within Codex, IDF has again reviewed the scientific literature; this work is described in the subsequent sections of this submission. Sections 2 and 3 provide information relevant to the questions raised by the CAC and CCNFSDU, respectively. In addition, for the convenience of interested parties, IDF has summarized the references to NCFs for soy protein as found in Codex standards (Appendix 1). Furthermore, Appendix 2 provides a brief summary of current developments relating to protein quality, which stress the importance of the actual levels of indispensable amino acids in individual protein sources.

2. REVIEW OF THE LITERATURE

2.1 Summary of IDF Bulletin 405

IDF Bulletin 405 [1] reviewed the literature relating to NCFs for a range of foodstuffs. It concluded:

There exists no scientific justification to support the change of the original protein source nitrogen conversion factor for soy from 5.71 to 6.25 ...

This conclusion was based on the assessment of scientific publications detailing experimentally determined NCFs from analysed samples and/or theoretical NCFs calculated from amino acid data (summarized in Table 1).

Table 1.NCFs cited in IDF Bulletin 405 [1] from studies on the NCF for soy protein, based on experimental analyses of samples and/or theoretical calculations based on amino acid data. In some cases, additional data from the original papers have been added for a more comprehensive overview

Product	NCF	References
Soy	5.71	[5]
Soy flour	5.71	[6]
Soy products	5.71	[6]
Soy	5.75–5.8	[7]
Soy isolate	5.6–5.8	[7]
Soy - commercial defatted flakes	5.66[a]	[8]
Soy - experimental acid precipitate isolate	5.77[a]	[8]
Soy - experimental dialysis isolate	5.80[a]	[8]
Soy - commercial isolate	5.70[a]	[8]
Soy meal	6.30[b] 5.65[c]	[9]
Soybean	k_A = 5.67[d] k_P = 5.38[e] k = 5.52[f] (the author claimed this to be the best estimate of the true NCF for soybean)	[10]

[a] Determined by the so-called "Factor Method" (dividing the sum of amino acid residue weights by the sum of their amino acid nitrogen content). A second method, called the "Kjeldahl Method" involved dividing the sum of amino acid weights by the micro-Kjeldahl nitrogen content of the foodstuff. The results obtained by the Kjeldahl Method were very

[1] The term "nitrogen conversion factor" may also be referred to by other terms such as "protein conversion factor" and "nitrogen-to-protein conversion factor".

similar to those obtained by the Factor Method namely 5.66, 5.76, 5.79 and 5.70 respectively. This study reported corrected values for those reported by the same author in 1981 [11], where subtraction of a water molecule from the amino acid molecular weight basis had been omitted, resulting in erroneously high estimates of nitrogen conversion factors as well as poor agreement between the results of the Factor Method and those of the Kjeldahl Method.
[b] Excluding nitrogen from asparagine and glutamine amide groups.
[c] Including nitrogen from asparagine and glutamine amide groups.
[d] Similar as the Factor Method, using the sum of anhydrous amino acid residues. Note that IDF Bulletin 405 [1] reported only a figure of 5.76 which appears to be a transcription error of the value of k_A.
[e] Similar principle as the Kjeldahl Method.
[f] The factor k can be estimated as follows: $k = (k_A + k_P)/2 \pm (k_A - k_P)/4$.

2.2 Recent review of the literature

In the current submission, IDF presents a new literature review focusing on papers dealing with the determination of NCFs for soy. This literature search yielded some papers from the period prior to 2006 that had not been mentioned in IDF Bulletin 405. In addition, several relevant papers published in the period 2006 to 2016 were found and are included in Table **2**.

Table 2. Additional NCFs for soy protein as reported in the literature, based on experimental analysis and/or theoretical computation

Foodstuff	NCF[a]	Comments	Reference
Soybean meal	5.69	Determined for crude protein	[12]
Soybean meal	5.71	Using the method of Tkachuk [12]	[13]
Soybean isolate	5.74	Using the method of Tkachuk [12]	[13]
Soybean	5.63	Determined for crude protein. Accounting for NPN reduced the NCF to a value of 5.22	[14]
Soybean/soybean meal	5.50	Average value calculated from data by Sarwar et al [15] (5.64), Mossé [10] (5.52), Tkachuk [12] (5.44) and Morr [8] (5.40)	[16]
Soybean meal[b]	k_A 5.64 k_P 5.13 k 5.39	NCF definitions based on Mossé [10]. Note that total N was determined according to the Dumas method instead of the Kjeldahl Method. Mossé [10] concluded that k gives the best estimate of the true NCF. Mariotti et al. [16] are of the opinion that k_A should be preferred for *purified* protein (where the amount of non-protein nitrogen (NPN) is low) such as purified protein products extracted from milk and soybean, but that k_P is preferred when assessing the protein content from the nitrogen content. However, it is noted that k_P depends on the analytical recovery during amino acid recovery and thus tends to underestimate the protein content. Sriperm et al. [17] conclude that k_A is the best estimate of the NCF for determining true protein.	[17]
Soy β– conglycinin (α’)	5.58[c]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [18]	[19]
Soy β– conglycinin (α)	5.65[c]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [18]	[19]
Soy β– conglycinin (β)	5.66[c]	7S protein subunit; calculated from protein structure based on data from Utsumi et al. [18]	[19]
Glycinins (mean value of the 5 subunits)	5.56[c]	11S protein; calculated from protein structure based on data from Utsumi et al. [18]	[19]
Soy cultivar 1	5.79[d]	Calculated for a cultivar having a ratio of glycinin (11S) to β-conglycinin (7S) of 0.5	[19]
Soy cultivar 2	5.73[d]	Calculated for a cultivar having a ratio of 11S to 7S of 1.0	[19]
Soy cultivar 3	5.69[d]	Calculated for a cultivar having a ratio of 11S to 7S of 1.5	[19]

[a] Figures rounded to two decimal places.
[b] Dehulled solvent extracted soybean meal; ingredient for animal feedstuffs.
[c] Calculated by excluding prosthetic groups.
[d] Calculated by including prosthetic groups.

2.3 Discussion of results reported in sections 2.1 and 2.2

2.3.1 What does the term “nitrogen conversion factor” denote?

It is important to realize that the term “nitrogen conversion factor” does not necessarily denote the same thing in different studies, because the methodologies used by different authors for determining NCFs are not necessarily the same. The issue as to what the term “nitrogen conversion factor” means is intimately linked to the question of what is meant by “protein”. This has been reviewed by Sosulski and Imafidon [20], Mariotti et al. [16] and Maubois and Lorient [19], and is likely to be the subject of further scientific debate. It is beyond the scope of the current review to repeat their considerations in detail here. However, the following is worth noting:

- Mariotti et al. [16] calculated two different NCFs:

  (1) *K*, for proteins *including* prosthetic groups;

  (2) *K'*, for proteins *excluding* prosthetic groups.

  Their rationale for computing *K'* was that *K* overestimates the potential of a source to provide amino acids when the peptide chains include prosthetic groups (glycosylated or phosphorylated residues).

  For soy products, applying the method proposed by Mariotti et al. [16] (i.e. to use *K'*) would reduce the NCF to a value of 5.50 (average of data from four different studies; see "Comments" in Table **2**), a reduction of about 4% compared with the traditionally used NCF of 5.71.

  However, Maubois and Lorient [19] point out that the prosthetic groups are constituent parts of the protein, because (1) they are covalently bound to the amino-acid chain, and (2) they possess nutritional, physiological and technological functions. Hence, they should be included in the calculation of the NCF.

- Whether or not to account for NPN in the calculation of the NCF for foodstuffs is a subject of debate. Accounting for NPN content will reduce the value of the NCF (for example, according to Sosulski and Holt [14] the NCF for soybeans reduces from 5.63 to 5.22 if it were corrected for NPN). However, Sosulski and Imafidon [20] advocate accounting for NPN, because the NPN fraction may contain substantial proportions of free amino acids and peptides.

2.3.2 What scientific evidence has been reported to support NCF=6.25 for soy?

To the best of IDF's knowledge, no study has been published in the scientific literature that calculated an NCF of 6.25 for commercial soy products from theoretical or experimental data.

Mossé [10] pointed out that an NCF factor of 6.25 is never valid in plant material. Indeed, the current review of studies covering soy products showed that the opinion of the various authors was very consistent in that they *rejected the use of a generic NCF of 6.25* (e.g. [8], [9], [10], [12], [14], [16], [17], [19]). Instead, all these authors advocated the use of specific NCFs based on scientific grounds[2], which agrees with the recommendation of FAO [21].

2.3.3. Are the NCFs for soy isolates and hydrolysates substantially different from those for other soybean products?

Commercial soybean products are classified into three major groups: (1) flour and grifts; (2) concentrates and (3) isolates having approximate protein levels of 40-54%, >70% and >90%, respectively, with the latter group supplying almost all the protein in liquid infant formulas [22]. Hence, it is worth assessing whether the NCFs for soy isolates and hydrolysates differ from those of other soybean products. Experimentally determined data obtained from studies in which different products were assessed are summarized in Table 3.

Table 3. Comparison of NCFs reported in the scientific literature for soy isolates/hydrolysates and other soybean products

Study	Product	NCF
Sosulski and Sarwar [13]	Soybean meal	5.71
	Soybean isolate	5.74
de Rham [7][a]	Defatted soy flour	5.66
	Soy isolate 1	5.85
	Soy isolate 2	5.64
	Soy isolate 3	5.63
	Soy hydrolysate 1	5.59
	Soy hydrolysate 2	5.56
Morr [8]	Defatted flakes	5.66
	Experimental soy isolate(acid precipitated)	5.76
	Experimental soy isolate (dialysis)	5.79
	Commercial soy isolate	5.70

[a] Experimental data from amino acid analysis, assuming 50% amidation

[2] This excludes foodstuffs containing blends of different proteins or of which the protein composition is unknown. In these cases the factor 6.25 has been proposed for practical reasons [20].

From the data in Table 3 it can be concluded that there is some variation in the estimates of the NCFs for soy isolates (range 5.63–5.85) and that the mean of 5.73 is very close to the average value (5.68) for all soy products reported in these respective studies. Furthermore, the mean NCF of 5.73 is also very close to that stated by Codex as appropriate for soy based infant formula (5.71) [2].

The data support the conclusion by Mossé [10] who studied the effect of different methodologies for determining NCFs and stated, "…the present work shows that k_A is close to 5.7 for soybean proteins and this value is the real conversion factor for purified soy protein isolates." Hence, from the scientific literature there is no evidence that the NCF for soy protein isolates is substantially different from that determined for other soy products. Therefore, it appears that the use of 6.25 for soy isolates results in an overestimation of the protein content by about 8–9%.

The limited data for soy hydrolysates (Table 3) suggest that also for this product type the NCF is similarly close to that of the defatted products, albeit somewhat lower. Again, the literature provides no scientific evidence to suggest that 6.25 is a justifiable factor for these products.

3. WHAT ARE APPROPRIATE NCFS FOR FORMULAS FOR INFANTS AND YOUNG CHILDREN?

In the Codex working paper CX/NFSDU 15/37/5-Add.1 *Review of the standard for follow-up formula (CODEX STAN 156-1987)* [23], both the Federation of European Specialty Food Ingredients Industries (ELC) and the European Vegetable Protein Federation (EUVEPRO) state:

*"In 1931 (revised in 1941), USDA scientist D.B. Jones published a report ("Circular 183")[1] which proposed establishing unique nitrogen to protein conversion factors for several foods. Jones reported 5.71 as a more "precise" factor for soy protein. In this Circular[1], Jones hypothesized that not all nitrogen in foodstuffs was protein nitrogen and not all proteins contained 16% nitrogen; therefore, a universal conversion factor of 6.25 was not always appropriate. In support of his theory, Jones reported nitrogen contents for several plant and animal proteins from a variety of sources. Jones justified the 5.71 factor for soybeans by stating, incorrectly, that the major protein in soybeans is glycinin, a globulin composed of 17.5% nitrogen. From these data, he designated a conversion factor for soy protein of 5.71 (100 divided by 17.5 results in a factor of 5.71). Glycinin (11S), however, represents only about 31-52% of the total protein in soybeans[2-4]. There are many other proteins in soybeans, including beta-conglycinin (7S), which represents about 35% of the total protein[2-4]. **If one considered only the 7S protein, the nitrogen to protein conversion factor for soy would be as high as 6.45[3,4]**. The ratios of 11S to 7S in soybeans will vary significantly, depending on the soybean variety and differences in seasonal growing conditions[2-4].*

[1] *Jones, DB (1931, slightly revised 1941) Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Protein. US Department of Agriculture Circular 183.*
[2] Murphy, PA and Resurreccion, AP (1984) Varietal and Environmental Differences in Soybean Glycinin and ß-Conglycinin Content. Journal of Agricultural Food Chemistry 32: 911-15.
[3] Roberts, RC and Briggs, DR (1965) Isolation and Characterization of the 7S Component of Soybean Globulins. Cereal Chem 42:71.
[4] Koshiyama, I (1968) Chromatographic and sedimentation behavior of a purified 7S protein in soybean globulin. Cereal Chem 45:405."

Roberts and Briggs [24] did not report an NCF value for the 7S fraction they isolated. However, they did report that the nitrogen content of the protein isolated by them was 15.5%. We assume that ELC and EUVEPRO used this figure to calculate the NCF value they quote (100/15.5=6.45). This value could be inaccurate because of underestimation of the nitrogen content and/or overestimation of the protein content.

Koshiyama [25] reported neither an NCF value nor nitrogen data that would allow calculation of the NCF. Hence, this publication provides no independent support for an NCF of 6.45 for the 7S fraction.

Furthermore, ELC and EUVEPRO omitted to contrast the figure derived from the study by Roberts and Briggs [24] with those of later and more detailed studies on soy protein structure, such as those reviewed by Maubois and Lorient [19]. The latter authors calculated that NCFs for the three 7S soy protein subunits (α', α and β, respectively) lie within a narrow range of 5.58–5.66 (mean 5.61) when excluding the prosthetic groups (Table **2**). But, as mentioned in section 0, prosthetic groups should be included, and if this is done then the mean NCF for the 7S protein becomes 5.91. Hence, by taking into account the covalently bound prosthetic groups, Maubois and Lorient [19] calculated that NCFs for different soy cultivars with different ratios of 11S to 7S (0.5, 1.0 and 1.5) lie in the range 5.69–5.79 (Table **2**). The mean value of 5.74 is very close to the 5.71 value stated in Codex Standard 72 [2], and clearly shows that factors of 6.45 and 6.25 would respectively overestimate the protein content in infant formula and follow-up formula by about 10–12% and 7–9%.

4. CONCLUSIONS

On the basis of a review of the scientific literature, it can be concluded that:

- The overwhelming consensus of scientific studies is that specific NCFs for specific foods should be used.
- 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. The use of NCF=6.25 overestimates the soy protein content by 8–9%.
- 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) does not appear to be 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 very close to the value of 5.71 stated in the Codex Standard for Infant Formula [2] as applicable to soy-based infant formula.
- The value of the NCF determined depends on whether or not:
    a) Glycosylated and/or phosphorylated prosthetic groups are included (excluding the prosthetic groups results in lower values for the NCF). It can be argued that these prosthetic groups are to be considered as constituent parts of the protein, because:
        - They are covalently bound to the amino acid backbone;
        - They have nutritional, physiological and technological functions.

    b) NPN is considered. An argument in favour of accounting for NPN when establishing NCFs is that the NPN fraction can contain substantial proportions of free amino acids and peptides.

5. REFERENCES

[1] IDF (2006) IDF Bulletin 405, Comprehensive review of scientific literature pertaining to nitrogen conversion factors. International Dairy Federation, Brussels.

[2] FAO/WHO (1981) Codex Standard 72, Standard for infant formula and formulas for special medical purposes intended for infants. FAO/WHO, Rome.

[3] FAO/WHO (2015) Joint FAO/WHO Food Standards Programme. Report of thirty-eighth session of Codex Alimentarius Commission, Geneva, Switzerland, 6-11 July 2015. REP15/CAC, Clause 22. Codex Alimentarius Commission, Rome. Available at http://www.codexalimentarius.org/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-701-38%252FReport%252FREP15_CACe.pdf.

[4] FAO/WHO (2015) Joint FAO/WHO Food Standards Programme. Report of thirty-seventh session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. REP16/NFSDU, Clause 57b. Codex Alimentarius Commission, Rome. Available at http://www.codexalimentarius.org/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-37%252FREP16_NFSDUe.pdf.

[5] Jones, D. B. (1941) Factors for converting percentages of nitrogen in foods and feed into percentages of proteins. Circular No. 183 (Original version, 1931). United States Department of Agriculture, Washington DC.

[6] FAO (1970). Amino acid content of foods and biological data on proteins. FAO Nutritional Study 24. FAO, Rome.

[7] de Rham, O. (1982) La proportion d’azote dans les protéines et le facteur de calcul protéine /azote. Lebensm. Wiss. Technol. 15: 226-231.

[8] Morr, C. V. (1982) Recalculated nitrogen conversion factors for several soybean protein products. J. Food Sci. 47: 1751-1752.

[9] Boisen, S. Bech-Andersen, S. & Eggum, B. O. (1987) A critical view on the conversion factor 6.25 from total nitrogen to protein. Acta Agric. Scand. 37: 299-304.

[10] Mossé, J. (1990) Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to see protein content. J. Agric. Food Chem. 38: 18-24.

[11] Morr, C. V. (1981) Nitrogen conversion factors for several soybean protein products. J. Food Sci. 46: 1362-1367.

[12] Tkachuk, R. (1969) Nitrogen-to-protein conversion factors for cereals and oilseeds meals. Paper No. 279 of the Grain Research Laboratory, Board of Grain Commissioners for Canada. Cereal Chem. 46(4):419-423

[13] Sosulski, F. W. & Sarwar, G. (1973) Amino acid composition of oilseed meals and protein isolates. Can. Inst. Food Sci. Technol. J. 6: 1-5.

[14] Sosulski, F. W. & Holt, N. (1980) Amino acid composition and nitrogento-protein factors for grain legumes. Can. J. Plant Sci. 60: 1327-1331.

[15] Sarwar, G. C. D. A., Finlayson, A. J., Friedman, M., Hackler, L. R., Mackenzie, S. L., Pellet, P. L. & Tkachuk, R. (1983) Inter- and intralaboratory variation in amino acid analysis of food proteins. J. Food Sci. 48: 526-531.

[16] Mariotti, F., Tomé, D. & Mirand, P. P. (2008) Converting nitrogen into protein – Beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. 48: 177-184.

[17] Sriperm, N., Pesti, G. M. & Tillman, P. B. (2011) Evaluation of the fixed nitrogen-to-protein (N : P) conversion factor (6.25) versus ingredient specific N: P conversion factors in feedstuffs. J. Sci. Food Agri. 91: 1182-1186.

[18] Utsumi, S., Matsumura, Y. & Mori, T. (1997) Structure-function relationships of soy proteins. In: Damodaran, S. & Paraf, A. (Editors) Food proteins and their applications, pp. 257-291, Marcel Dekker, New York.

[19] Maubois, J.-L. &Lorient, D. (2016) Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. J. Dairy Sci. Tech. (in press). doi: 10.1007/s13594-015-0271-0.

[20] Sosulski, F. W. & Imafidon, G. I. (1990) Amino Acid Composition and Nitrogen-to-Protein Conversion Factors for animal and plant foods. J. Agric. Food Chem. 38: 1351-1356.

[21] FAO (2003) Food energy – methods of analysis and conversion factors. FAO Food and Nutrition Paper 77, FAO, Rome.

[22] Singh, P., Kumar, R., Sabapathy, S. N. & Bawa, A. S. (2008) Functional and edible uses of soy protein products. Compr. Rev. Food Sci. Food Saf. 7: 14-28.

[23] FAO/WHO (2015). Joint FAO/WHO Food Standards Programme. Thirty-seventh session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), Bad Soden am Taunus, Germany, 23-27 Nov 2015. Review of the Standard for Follow-Up Formula (Codex Stan 156-1987). CX/NFSDU 15/37/5-Add.1, 2015. Available at: http://www.codexalimentarius.org/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-37%252FWD%252Fnf37_05-Add.1e.pdf .

[24] R Roberts, R. C. & Briggs, D. R. (1965) Isolation and characterization of the 7S component of soybean globulins. Cereal Chem. 42: 71-85.

[25] Koshiyama, I. (1968) Chromatographic and sedimentation behaviour of a purified 7S protein in soybean globulins. Cereal Chem. 45: 405-412.

[26] Lewis, J. L. (2012) The regulation of protein content and quality in national and international food standards. Brit. J. Nutr. 108 (Suppl. S2): S212-S221.

[27] Moughan, P. (2012) Dietary protein for human health. Brit. J. Nutr. 108 (Suppl.S2): S1-S2.

[28] FAO (2013) Dietary protein quality evaluation in human nutrition," Report of the Expert Protein Consultation. Report of an FAO Expert Protein Consultation Rome. FAO Food and Nutrition Paper 92. FAO, Rome.

[29] Gilani, G. S. (2012) Background on international activities on protein quality assessment of foods. Brit. J. Nutr. 108 (Suppl. S2): S168-S182.

[30] Schaafsma, G. (2000) Criteria and significance of dietary protein sources in humans. J. Nutr. 130: 1865S-1867S.

[31] Schaafsma, G. (2012) Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. Brit. J. Nutr. 108 (Suppl. S2): S333-S336.

APPENDIX 1 **References to NCFs for soy within Codex standards**

Standard	Codex Committee	Title	Section	Relevant provision
CODEX STAN 72 – 1981	CCFNSDU	Standard For Infant Formula And Formulas For Special Medical Purposes Intended For Infants	3.1.3 (a) Protein, Footnote 2	For the purpose of this standard, the calculation of the protein content of the final product prepared ready for consumption should be based on **N x 6.25**, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The protein levels set in this standard are based on a nitrogen conversion factor of 6.25. The value of 6.38 is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of **5.71** as a specific factor for conversion of nitrogen to protein in other soy products.
CODEX STAN 175-1989	CCVP	Codex General Standard For Soy Protein Products	2 – Descrip-tion	Soy Protein Products (SPP) covered by this standard are food products produced by the reduction or removal from soybeans of certain of the major non-protein constituents (water, oil, carbohydrates) in a manner to achieve a protein **(N x 6.25**) content of:
			3.2.2	Crude protein (**N 6.25**) shall be: – in the case of SPF, 50% or more and less than 65% – in the case of SPC, 65% or more and less than 90% – in the case of SPI, 90% or more on a dry weight basis excluding added vitamins, minerals, amino acids and food additives.
CODEX STAN 234-1999	CCMAS	Recommended Methods Of Analysis And Sampling		• Soy protein products – Protein - AOAC 955.04D (**using factor 6.25**) - Titrimetry , Kjeldahl digestion • Infant formula - Crude protein[1] - ISO 8968-1 | IDF 20-1 - Titrimetry (Kjeldahl) *1) Determination of Crude Protein* *The calculation of the protein content of infant formulas prepared ready for consumption may be based on **N x 6.25**, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The value of 6.38 is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of **5.71** as a specific factor for conversion of nitrogen to protein in other soy products* • Non-fermented soybean products - Protein content - NMKL 6 or AACCI 46-16.01 or AOAC 988.05 or AOCS Bc 4-91 or AOCS Ba 4d-90 (**Nitrogen factor 5.71**) - Titrimetry, Kjeldahl digestion • Tempe - Protein content - NMKL 6 or AOAC 988.05 or AACCI 46-16.01 (**Nitrogen factor 5.71**) - Titrimetry, Kjeldahl digestion
CODEX STAN 298R-2009	CCASIA	Regional Standard For Fermented Soybean Paste (Asia)	Note 2 to Table in 3.2	The **nitrogen conversion factor of 5.71** should be used.
CODEX STAN 313R-2013 Amendment 2015	CCASIA	Regional Standard For Tempe (Asia)	8 – Methods of sampling and analysis	Protein Content: NMKL 6, 2004 or AOAC 988.05 or AACCI 46-16.01 (**Nitrogen factor 5.71**)
REP15/ASIA APPENDIX IV	CCASIA	Draft Regional Standard For Non-Fermented Soybean Products (Step 8)	9.1.2 Determination of Protein Content	According NMKL 6, 2004 or AACCI 46-16.01 or AOAC 988.05 or AOCS Bc 4-91 or AOCS Ba 4d-90, **nitrogen factors for non-fermented soybean products are 5.71**.

APPENDIX 2 PROTEIN QUALITY

Protein is an important nutrient and must deliver all the indispensable amino acids in the correct balance for growth and maintenance. The protein content of a food is not the only criteria for adequate human nutrition; increasingly regulators [26], food industry and health care professionals will recognise the relevance of protein quality.

Protein quality refers to the ability of the amino acids in foods to adequately meet human requirements for indispensable amino acids. Amino acid requirements vary for specific age groups and physiological conditions [27]. The consequences of inadequate protein intake to meet indispensable amino acid requirements are well known and include stunted growth, increased susceptibility to infection, suboptimal muscle capacity and diminished mental performance (from retardation to apathy). A precise assessment of the ability of a dietary protein source to match the body's needs for individual amino acids will allow better use of an increasingly scarce resource [28].

What is PDCAAS and DIAAS?

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a simple and widely used methodology for evaluating protein quality. PDCAAS is derived from the ratio between the first limiting amino acid in the protein and its corresponding value from the amino acid reference pattern, and corrected for true faecal nitrogen (N) digestibility.

PDCAAS may be acceptable for the routine evaluation of the digestibility of protein from mixed human diets containing high quality protein sources. However, it may be inappropriate for evaluating protein quality of foods that are a major proportion of the diet, *e.g.* infant formula, enteral products, or novel or supplementary foods that contain anti-nutritional factors [28].

Furthermore, the PDCAAS has limitations [29-31]:

- PDCAAS values are truncated to 100%, or 1, which limits high quality proteins relative to poorer quality proteins and fails to recognize the advantages of surplus amino acids to complement poorer quality proteins in mixtures. Truncation removes any nutritional differences between high protein foods such as milk and soy, although actual concentrations of important dietary indispensable amino acids, which may be limiting in some diets, are higher in milk than in soy. This could be recognized by giving individual protein sources an amino acid score of > 1 (or > 100).
- Faecal N digestibility likely overestimates the delivery of dietary amino acids to the body.
- Anti-nutritional factors in plant proteins or processed foods may lead to higher endogenous amino acids losses. Thus PDCAAS may inappropriately reflect high scores.
- The amino acid reference pattern used is based on minimum requirements for growth and maintenance using the pattern for 2-5 year old children and does not reflect optimal intake.

Hence, the recent FAO Expert Consultation "Dietary protein quality evaluation in human nutrition" recommends a new, advanced method, the Digestible Indispensable Amino Acid Score (DIAAS) for assessing the quality of dietary proteins [28]:

$$\text{DIAAS} = \frac{\text{mg of digestible dietary indispensable amino acid in 1 g of the dietary protein}}{\text{mg of the same dietary indispensable amino acid in 1 g of the reference protein}}$$

The FAO are convening expert groups to review research needs and to develop a programme of work that will address the above questions and create the data needed to gain formal FAO endorsement of the DIAAS method.