CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 2 **MAS/37 CRD/8**

ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS SAMPLING

Thirty-seventh[th] Session
Budapest, Hungary, 22 – 26 February 2016

MATTERS REFFERED BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER CODEX COMMITEES

Nitrogen conversion factor for soy protein

(comments submitted by Ecuador, India, Kenya, Nigeria, ENSA, EUVEPRO)

ECUADOR

El laboratorio oficial del país, el Laboratorio de Referencia de la Agencia Nacional de Regulación, Control y Vigilancia Sanitaria ARCSA, utiliza para el cálculo de la proteína en alimentos de soya el factor 5.71, basado en los factores recomendados por la FAO/OMS (1973), fundamentado en los perfiles de aminoácidos de la FAO/OMS y sus consideraciones para las proporciones de amidas presentes.

INDIA

Para 6: The conversion factor for Nitrogen to protein is 5.71 as per IS 7219:1973 RA 2005, it is opined that the use of a single factor, 6.25, is confused by two considerations. First, not all nitrogen in foods is found in proteins: it is also contained in variable quantities of other compounds, such as free amino acids, nucleotides, creatine and choline, where it is referred to as non-protein nitrogen (NPN). Only a small part of NPN is available for the synthesis of (non-essential) amino acids. Second, the nitrogen content of specific amino acids (as a percentage of weight) varies according to the molecular weight of the amino acid and the number of nitrogen atoms it contains (from one to four, depending on the amino acid in question). Based on these facts, and the different amino acid compositions of various proteins, the nitrogen content of proteins actually varies from about 13 to 19 percent. This would equate to nitrogen conversion factors ranging from 5.26 (1/0.19) to 7.69 (1/0.13).

Further to this, the conversion factor of 5.71 for soybean products in general is supported by scientific literature (see Table below) and should be retained in view of the scientific understanding that a specific factor for converting nitrogen content into protein should be employed where available.

Table: Some references on scientifically analyzed samples of soy protein source

Ref No.	References : scientific publication, analytical data, international standard	Product Name/Class	Nitrogen Conversion Factor (NCF)	% N in Protein
1	De Rham, O. (1982) Lebensm. Wiss. Technol. 15, 226-231.	Soy Isolate Soy (Glycine max	5,6-5,8*** 5,75-5,8***	17,54 17,24
2	Boisen, S. Bech-Andersen, S.and Eggum B.O. (1987) Acta Agric.Scand. 37, 299-304	Soy Meal	5,65*** With Amides	17,70
3	Mosse, J. (1990) J. Agric. Food Chem. 38, 18-24.	Soy (Glycine max)	5,38-5,67***	18.18
4	Jones, D.B. (1941) United States	Soy (Glycine max)	5,71	17,51

	Department of Agriculture, Circular No. 183. (Original version 1931).			
5	FAO/WHO (2003) FAO food and nutrition paper 77, Rome, ISSN 02544725	Soy (Glycine max)	5,71	17,51
6	Leatherhead Food Research Association. Analytical Methods Manual. 1996. Nitrogen (or Total Protein) Content by Kjeldahl.	Soy (Glycine max)	5,71	17,51
7	FAO/WHO (1970) FAO Nutritional Study 24, Rome	Soy (Glycine max) Soy Flour Soy Products	5,71 5,71 5,71	17,51 17,51 17,51
8	AOAC 945.39	Soy Flour	5,70	17,54

Note: * Calculated from amino acid data

Rationale: Presently there exists no scientific justification for change of original protein source nitrogen conversion from 5.71 to 6.25. The protein content is completely dependent on the total nitrogen available in soya products and the use of 6.25 will have a disadvantage as either 5.71 or 6.25 are meagre conversion factor and the actual nitrogen present in a product goes on diet hence suggestion to 6.25 may give an advantage to industry to produce products with low nitrogen content and by virtue of a conversion factor the total protein content is enhanced. Change in this conversion factor should be scientifically and judicially dealt before concluding.

KENYA

Kenya agrees to the use of the conversion factor of 5.71 to determine the protein content in soybean products.

NIGERIA

Nigeria supports the assessment and appropriate use of protein content soybean products in general content the country is involved in the production and consumption of soybean products.

EUROPEAN NATURAL SOY AND PLANT BASED MANUFACTURERS ASSOCIATION (ENSA)

Introduction

ENSA is the European Natural Soy and Plant Based Manufacturers Association that represents the interests of soy product manufacturers in Europe. Soy products marketed by ENSA members are intended for human consumption, 100% plant-based and manufactured **from the whole soybean, using a natural process**: soybeans are soaked in water, milled and other ingredients and components may be added e.g. vitamins and minerals. The products contain the natural nutrients of the soybean, such as high quality soy protein.

As a key stakeholder and Codex Observer we would hereby like to comment on the NCF (Nitrogen Conversion Factor) of soy protein which will be discussed in the 37th session of the Codex Committee on Methods of Analysis and Sampling(CCMAS) (Budapest 22-26 February 2016).

We are concerned that a nitrogen protein conversion factor (NCF) of 5.71 for soyprotein (as proposed in the information sheet distributed by the IDF), instead of the widely accepted NCF of 6.25 is not in line with the globally recognized standards of the Codex Alimentarius and Analytical Sciences Associations(AOAC), national and regional governmental nutrition and labelling regulations. A change of the NCF would also have a significant impact on the recognition of soyfoods as nutritious and high-quality protein source.

Labeling of protein in EU and beyond

Protein is an important component of the diet playing an essential role as structural and functional components of living organisms. Dietary protein provides amino acids which serve as building blocks for protein synthesis necessary for all vital organs, muscles, hormones and biological fluids such as blood.

For the calculation of protein content of food the Kjeldahl method has been used since 1880. The method requires a Nitrogen to Protein conversion factor which was initially determined to be 6.25 for all food products. The 6.25 NCF for soy protein is supported by international consensus of scientific and regulatory experts and organizations.

In the EU regulation 1169/2011 on the provision of food information to consumers Annex I, it is stated that the labeling of protein should be defined as the protein content calculated using the formula ‘protein= Kjeldahl nitrogen x 6.25 ‘.

The recently published Commission delegated Regulation (EU)2016/127 of 25 September 2015 supplementing Regulation 609/2013 regarding compositional requirements of infant formula and follow-on formula also mentions in Annex 2 ‘protein content = nitrogen content x 6.25.

The nutrition labeling regulation of many countries apply a NCF of 6.25 (including US, Argentina, Brazil, Mexico, and South Africa).

Background

The NCF for soy of 5.71, proposed for discussion at the 37th session of the Codex committee on Methods of Analysis and Sampling, is based on **outdated and inaccurate data** from a paper published in 1931 by USDA scientist D.B. Jones. Jones justified this factor by stating incorrectly that major protein in soybeans is glycinin composed of 17.5% nitrogen (100:17.5= 5.7); however glycinin only represents about one third of the total protein in soybeans.

There are many other proteins in soybean including beta-conglycinin which represents about 35% of the protein. If only beta-conglycinin would be taken into account the NCF would be 6.29 - 6.45.

Growing conditions of the soybean is responsible for variation in protein ratios.

Current knowledge

Today there is a general agreement that the calculation of the ‘true’ nitrogen to protein conversion factors is difficult as it requires a thorough knowledge of the structure of all proteins, which is not available for most proteins, including soy.

Therefore, no solid conclusions can currently be made based on the available scientific research. A recent publication of Maubois (dairy research Lab at Inra, France) published in a magazine related to dairy research (Dairy Science and Technology), does not provide any additional scientific data to support a change of NCF of 5.7 and as such the globally recognized NCF for soy, milk and other proteins should remain 6.25. The authors point out that precise analytical determination faces numerous difficulties inherent to the methods used and also lack precision and certainty.

For calculation of the NCF values of soy protein, the authors claim that they used the sequence data mentioned in a publication of Utsumi (1992); however it is unclear how the data have been calculated.

Soy protein: high quality protein

Furthermore, soyfoods have been shown to be good sources of high quality protein. Soy protein is well digested and has an amino acid pattern that matches well the human biological requirements. Human nutrition research continues to demonstrate that soy is a high quality protein that supports growth and maintenance when consumed as a sole source protein. PDCAAS values of soy protein range from 0.9 to 1.0, which is similar to animal proteins such as egg, meat and dairy (Hughes 2011).

Nitrogen balance studies found that soy protein is comparable to milk and meat in its ability to maintain N balance in the body (Rand 2003).

Conclusion

- The 5.71 conversion factor for soy protein, based on Jones’s logic, is incorrect.
- There is no scientific basis for changing the globally recognized NCF for soy protein from 6.25 to 5.71
- There is no consistency in calculation for determination of NCF’s of different type of protein.
- When discussing the NCF all type of protein needs to be reconsidered, including dairy.

Therefore, ENSA request the continued use of NCF of 6.25 for soy protein.

Harmonization of nutritional labelling and product standards across professional organization and governments is best served by using the 6.25 NCF.

References

Hughes, G.J et al.(2011) Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) for Soy Protein Isolates and Concentrate: Criteria for Evaluation. J Agric Food Chem.

Rand et al. (2003) Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. Am J clin nutr 77(1):109-127

EUROPEAN VEGETABLE PROTEIN ASSOCIATION (EUVEPRO)

EUVEPRO is the European Vegetable Protein Association, representing the interests of manufacturers and distributors of vegetable proteins for human consumption (food) in the European Union.

As Codex Observer we would hereby like to submit comments on

- *the assessment of the appropriateness of the use of the conversion factor of 5.71 to determine protein content in soybean products in general*
- *the accuracy and appropriateness of 5.71 as the nitrogen factor for soy protein isolates used in formula for infants and young children and to take into account the amino acid profile of the isolate*

EUVEPRPO calls for the continued use of N x 6.25 for the determination of soy protein content. We are concerned that the proposed use of N x 5.71 as the nitrogen to protein conversion factor for soy instead of the widely accepted N x 6.25 factor represents a departure from:

1. Current Codex Standards 175-1989 Codex General Standard for Soy Protein Products, 174-1989 “Codex General Standard for Vegetable Protein Products, and CAC/GL 2-1985 “Guidelines on Nutrition Labelling” (as amended by the 29th Session of the Commission, 2006),
2. The guidance of globally recognised scientific organisations such as AOCS, AACC and ISO, and
3. Member country government regulations (for example, Argentina, Brazil, China, the European Union, India, Japan, Korea, and the United States).

The change to N x 5.71 would have a significant negative impact on the perception of soy as a nutritious and high-quality protein: It results in an almost 10% reduction in the calculated protein content of soy products without any change to the composition of the product. This would have serious repercussions on isolated soy protein as a food ingredient – it would no longer meet certain product requirements, may disappear from ingredients lists, could result in expensive formula changes, and create significant extra costs to manufacturers due to the resulting changes to the food labels.

One example of a food category issue would be soy protein based infant and follow-on formulae, for babies and children that cannot tolerate mother’s or cow’s milk, which in the European Union can only be derived from soy protein isolates. With a change in protein conversion factor to 5.71, soy protein isolates will no longer meet the Codex Standard and as a result, this category of foods could no longer be produced.

Also, and very particularly, the choice of 5.71 as the conversion factor for soy proteins is based on old analytical data that (a) only recognizes some of the protein moieties that are actually present in soy beans, and (b) is based on Kjeldahl and combustion methods, rather than the more accurate analysis of amino acids that is currently recommended (FAO, 2003, for example).

The calculation of the amount of protein in foods is typically performed using the conversion factor N x 6.25 which allows for international harmonisation in the expression of protein levels. A move towards unique factors for dairy proteins and soy proteins would, for consistency, also require the definition and application of unique conversation factors for all dietary proteins – a burdensome, costly and, from a trade and regulatory angle, impractical exercise.

EUVEPRO appreciates the very detailed AOCS “Position on the Nitrogen Conversion Factor for Soy Protein” and the provision in this document of data on the use of amino acid contents as a basis for more accurate determinations of conversion factor for soy protein. EUVEPRO fully supports the comments made by AOCS in this paper.

Scientific and Regulatory Arguments

<u>What is the origin of 6.25 ?</u>

The Kjeldahl method, the modified Kjeldahl method, and the combustion method (known as the Dumas method) are commonly used for analytical measurement of protein. These methods measure protein in foods indirectly by assessing the quantity of nitrogen that can be released from a protein and captured as ammonia. Nitrogen from all nitrogenous compounds, including proteins and non-protein material, are typically included in this total. In the early 1880s, when the Kjeldahl method was invented, proteins readily available for testing (serum albumin and globulin from blood, casein from milk) contained about 16% nitrogen. Dividing 100 by 16% gave a nitrogen conversion factor of 6.25 and it was believed that this factor applied to all proteins. Although it has since been discovered through further scientific research that few foods contain precisely 16% nitrogen, use of the 6.25 conversion factor for measurement of protein sources has been maintained to allow for a measure of international harmonization in the expression of protein levels.

What is the origin of 5.71?

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].

What is the Support for 6.25

The 6.25 nitrogen conversion factor is recognized by Codex Alimentarius as the appropriate conversion factor for determining the protein content of a soy product per the following Codex Standards:

- Codex Standard 175-1989 Codex General Standard for Soy Protein Products[5]
- Codex CAC/GL 2-1985 Guidelines on Nutrition Labelling (as amended by the 29th Session of the Commission, 2006)[6]
- Codex Standard 234-1999 Recommended Methods of Analysis and Sampling (as amended by the 30th Session of the Commission, 2007)[7]

Although an exhaustive list of regulations from around the globe was not assessed, the nutrition labeling regulations or regulatory product composition standards for the following countries representing a significant portion of the world's population list 6.25 as the N conversion factor for soy protein:

- Select National and Regional Government Nutrition Labeling Regulations
  - Argentina[8]
  - Brazil[9]
  - China[10] (for soy protein ingredients, isolated soy protein & soy protein concentrate)
  - European Union[11]
  - India[12]
  - Japan[13]
  - Korea[14]
  - Malaysia[15]
  - Mexico[16]
  - South Africa[17]
  - United States[18]

The following globally recognized analytical sciences associations identify 6.25 as an appropriate nitrogen conversion factor for soy in their current official analytical methods:

- American Oil Chemists Society (AOCS)[19-22]
- AOAC[23]
- AACC International (AACC)[24-27]
- International Organization for Standardization (ISO)[28]

Soy is a Source of High-Quality Protein

In addition, soy is a source of high quality plant protein, comparable to meat, milk, and eggs. Numerous nitrogen balance studies found soy protein is comparable to milk and meat in its ability to support N balance[29-34]. The 6.25 nitrogen to protein conversion factor was used by researchers to calculate gram amount for **both** soy and animal-based protein fed to study subjects. Rand, et al., 2003[35] conducted a meta-analysis of nitrogen balance studies that was used to estimate protein requirements for healthy adults and found soy protein is comparable to milk and meat in its ability to support nitrogen balance. Rand et al. stated, "These original soy studies showed clearly that the well-processed soy proteins were equivalent to animal protein, whereas wheat proteins were used with lower efficiency than were animal protein (beef)"[35].

The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is the currently accepted and validated method for protein quality measurement based on the principle that the nutritive value of a protein depends on its ability to provide amino acids in adequate amounts to meet the requirements of children and adults[36]. The PDCAAS for isolated soy protein and soy protein concentrate is equal to 1.0, comparable to milk and egg proteins[37,38].

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