CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

E

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

Agenda Item 2 **MAS/37 CRD/7**

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 AOCS)

Nitrogen conversion factor for soy protein

Table of Contents

List of Tables

I. Executive Summary

We hereby submit that the 6.25 nitrogen conversion factor for soy protein is supported by international consensus of the following scientific and regulatory experts and organizations:

- Codex Alimentarius
  - Codex Standard 175-1989 Codex General Standard for Soy Protein Products
  - Codex Standard 174-1989 Codex General Standard for Vegetable Protein Products (VPP)
  - Codex CAC/GL 2-1985 Guidelines on Nutrition Labelling (as amended by the 29th Session of the Commission, 2006)
  - Codex Standard 234-1999 Recommended Methods of Analysis and Sampling (as amended by the 30th Session of the Commission, 2007)
- National and Regional Government Nutrition Labeling Regulations
  - Argentina
  - Brazil
  - China
  - European Union
  - India
  - Japan
  - Korea
  - United States
- Globally Recognized Analytical Sciences Associations
  - American Oil Chemists Society (AOCS)
  - AOAC International (AOAC)
  - AACC International (AACC)
  - International Organization for Standardization (ISO)

The proposed 5.71 nitrogen conversion factor for soy protein is based on outdated and inaccurate data originally reported in 1931. These data have since been discredited with improvements in analytical methods and technology, as well as an increased understanding of the chemical composition of proteins and the effects of amino acids and protein on human health:

- Analytical data of amino acids for over 50 samples of various soy products conducted by the United States Department of Agriculture, independent laboratories, and an independent university researcher show a nitrogen conversion factor in a range of 6.24-6.37

Furthermore, the literature exploring approaches to calculating nitrogen to protein conversion factors present inconsistent outcomes, highlighting the uncertainties with trying to establish a “precise” conversion factor. Human nutrition research, however, continues to demonstrate that soy is a high-quality protein that supports growth and maintenance when consumed as a sole source protein and 6.25 is used to calculate the protein content of diets.

Changing the nitrogen conversion factor for soy protein from 6.25 to 5.71 will represent a departure from internationally recognized analytical methods, established nutrition clinical research procedures, as well as widely embraced trade and regulatory practices. Changing from the 6.25 to 5.71 conversion factor will result in an almost 10% reduction in the calculated protein content of soy foods without any change to the product itself. Potential impacts include:

- Elimination of isolated soy protein as a food ingredient from the marketplace as it will be impossible to meet the product standard 90% protein minimum using a 5.71 nitrogen to protein conversion factor
- Significant costs to food manufacturers due to expensive label changes
  - “Isolated soy protein” would have to be removed from product ingredient lists
  - Changes to protein nutrition labeling

  - Potential requirement for product formula changes
- Confusion for food manufacturers seeking to make products containing isolated soy protein
- Confusion for consumers seeking products containing isolated soy protein
- Impacts on presentation and interpretation of data from nutritional research for both scientific and lay audiences (which generally use 6.25)
- Significant cost increases for animal production facilities using soy as source of protein in feed rations

We therefore, support the continued use of the 6.25 nitrogen conversion factor for the measurement of protein in soybeans and soy products.

II. Introduction

We hereby submit that the 6.25 nitrogen conversion factor (NCF) for soy protein is supported by international consensus of scientific and regulatory experts and organizations. The World Health Organization (WHO) and the Food & Agriculture Organization of the United Nations (FAO)[1-5], as well as several national and regional governments recognize the 6.25 NCF for soy protein for purposes of trade, nutritional labeling, and the promotion of public health. The proposed 5.71 conversion factor is based on outdated and inaccurate data originally reported in 1931 by D.B. Jones, a USDA researcher[6]. These data were based on the 1898 publication of Osborne and Campbell[7] whose report did not claim that their values represented the nitrogen content of the whole bean, merely the fraction that they isolated. The Jones' factor of 5.71 has been disputed by other researchers who cite improvements in analytical methods and technology, as well as an increased understanding of the chemical composition of proteins[8-11] and the effects of amino acids and protein on human health. Changing the NCF for soy protein from 6.25 to 5.71 will represent a departure from internationally recognized analytical methods, established nutrition clinical research procedures, as well as widely embraced trade and regulatory practices. This position document will cover four important viewpoints that support a 6.25 NCF for soy, namely: published literature covering proposed approaches to calculating NCFs and human nutrition studies assessing a source of protein's impact on human health, the scientific analytical environment, analytical data on a variety of soy products based on a direct method of analysis recommended by the FAO (2003) for the measurement of protein[5], and the current regulatory environment.

In response to the proposal to explore the appropriate NCF for soy, we request a definition of the need to change the NCF: what pressures, scientific or economic, are driving the need for a new conversion factor? What public health or other benefits will justify the significant investment in time and money required to conduct this exploration? Further, if the consensus is that there is a critical need to conduct further assessment of the appropriate NCF for soy protein, in the interests of protecting the health of consumers, we believe the same exercise should be conducted for **all** commonly consumed proteins and the results of this work should be released and implemented into the appropriate Codex Standards simultaneously to ensure the ensuing impact on all proteins will be equally felt. To this end, a recent publication by Angell, et al., 2016[12] made the case that a specific NCF should be made for all seaweed products, and that doing so when the seaweed industry (as a protein source) is in its infancy will prevent potential economic losses (obviously not to the seaweed industry but more so to protein ingredient competitors), since they recommend a value lower than 6.25 for seaweed. Koletzko and Shamir[13] noted, in a commentary about a standard for infant formula, that a newsletter from the German dairy industry suggested that "the application of a NCF of 6.25 instead of 6.38 for all dairy products would lead to a loss of some €80m" for the dairy industry in Europe alone". There will be increasing pressure, then, in the face of increased efforts to introduce novel dietary protein sources to the global commercial market[14] to develop new NCFs for these proteins. Therefore, it is imperative that a global consensus as to how to measure protein for all human dietary proteins be established rather than to continue to depend on efforts driven by disparate motivations to derive NCFs which has led to different methods and approaches.

The critical nature of establishing a consensus on the procedure to calculate NCFs is most evident in the recently published Standard Tables of Food Composition in Japan (STFCJ) 2015[15] where none of the NCFs calculated by sum of the anhydrous amino acids for any of the foods were equal to 6.25. In fact, virtually all the foods measured by this method were significantly lower than 6.25, including dairy proteins. Thus, while the currently commonly used 6.25 NCF may be erroneous, it is equally erroneous for **all** proteins. In fact, this was noted by Marriotti, et al., 2008[16] and a ***corrected default value for all proteins of 5.6 was proposed.***

The Kjeldahl method, the modified Kjeldahl method, and the combustion methods continue to be widely used for analytical measurement of protein. Direct analysis of amino acids to quantitate protein, however, provides more accurate and nutritionally relevant values. We believe devoting time and resources to the validation of improved methods for measurement of protein, such as direct analysis of amino acids discussed in FAO Food and Nutrition Paper 77[5], and the dissemination of these data for public use would be more

aligned with the Codex Alimentarius Commission's Procedural Manual[17] on determining priorities and initiating new work than initiating work to determine the "precise" NCFs for widely consumed proteins.

III. Published Literature Relevant to Nitrogen Conversion Factors

A review of published literature exploring approaches to calculating NCFs was published in 2006 by the International Dairy Federation[18]. The papers published in this review present inconsistent outcomes, highlighting the uncertainties with trying to establish a "precise" NCF (Table 1). Many of the papers do not deal with the issue of non-protein nitrogen and investigators disagree as to what constitutes non-protein nitrogen. Some investigators believe that amino acids and peptides account for non-protein nitrogen[19] while others believe these should be considered as part of the protein content since the purpose of the developing these calculations are for nutritive purposes and all organisms utilize proteins in their hydrolyzed form of amino acids and peptides[16, 20].

Table 1. Publications on Methods to Calculate Nitrogen Conversion Factors

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
Osborne TB and Campbell GF (1898) J Am Chem Soc 20: 419-428[7]	Soy (Glycine hispida)	This paper did not propose a N to P conversion factor	17.5%	The authors of this paper did not claim that their values of %N represented the nitrogen content of the whole soybean, merely the fraction(s) that they separated; the authors claimed that glycinin was the major protein in the soybean but did not state the percent of glycinin typically found in soybeans Although not specifically cited by Jones, 1941[6] it is evident that Jones used this paper to arrive at the 5.71 NCF for soy
Jones DB (1941) United States Department of Agriculture, Circular No.183 (Original version 1931)[6]	Soy (Glycine max)	5.71	17.51	This citation bases the NCF for soy protein on the nitrogen content of only one of the storage proteins (glycinin),presumably based on the 1898[7] report above While citing 5.71 for soy protein, the Jones paper does not provide any data to show how this calculation was derived; only 1 sentence in the report is dedicated to soy protein The NCF for other crops is discussed in more detail but the IDF report[18] does not cite the Jones paper for the following crops: wheat (5.83), rye (5.83), barley (5.83) or oats (5.83)
Tkachuk R (1969) Cereal Chem 46: 419-423[20]	Defatted soybean	5.69		This paper derives NCFs for cereals and oilseeds based on data published in an earlier publication (Tkachuk, 1969 Cereal Chem 46: 206-218[21]) and derives glutamine and asparagine values from the content of ammonia (assumes all ammonia is derived from these 2 amino acids and simply divides the total ammonia by 2 and assigns the resultant values to asparagine and glutamine); this is based on Tkachuk's 1966 work in wheat (Tkachuk, 1966 Cereal Chem 43: 207-222[22]) where glutamine and asparagine values are directly measured by comparing enzymatically digested protein to acid hydrolyzed wheat protein; it is on this work *alone* in wheat that the assumption that free ammonia only comes from asparagine and glutamine; note that in the work on wheat, accurate estimates of the relative proportions of asparagine or glutamine were possible by direct measurement; errors would have resulted in NCF if one assumed equal proportions of both amino acids as subsequent investigators have done who cite this method Note also that this paper the author points out the errors and assumptions made in the Jones 1941[6] paper (i.e. not accounting for non-protein nitrogen), calling into question the NCF proposed by Jones[6]

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
DeRham O (1982) Lebensm. Wiss. Technol 15, 226-231[23]	Soy Isolate Soy (Glycine max)	5.6-5.8 5.75-5.8	17.54 17.24	DeRham points out that amino acid analytic methods do not routinely measure asparagine and glutamine, so in his analysis he ***assumed*** 50:50 or 75:25 amide:acid ratios when calculating the conversion factors from the listing of amino acid compositions of food in the FAO 1970 report[24] Soy protein has a ratio closer to 25:75 which would raise the calculated conversion factor from what deRham actually calculated DeRham points out that other investigators may have used different assumptions of amide:acid ratios (e.g. Jones 1941[6] and Morr 1981[9]) which may explain why conversion values in his report differ from those DeRham also questions Jones' stated values (Jones 1941[6]) and mentions that Jones used an arbitrary method to establish some of the conversion factors; DeRham also suggests that there are some errors in the Jones report, e.g. deRham suggests the conversion values reported in Jones 1941[6] for wheat flour and wheat bran should be inverted DeRham concludes his paper by saying that nutritional studies should continue to use the traditional 6.25 conversion factor until more precise conversion factors are available
Morr CV (1982) J Food Sci 47, 1751[25]	Soy (Glycine max)	5.76	17.36	Morr's 1982 paper is a follow-up of his 1981 paper (Morr, 1981 J Food Sci 46, 1362[9]); follow-up was in response to personal communications Morr received from Posati and de Rham and the follow-up paper was to try to "minimize the magnitude of the discrepancies within the N conversion factors" determined by the Kjeldahl method and Factor Method (the latter was proposed by Morr, 1981[9] and involves calculating the NCF based on residual weights of amino acids determined by amino acid analyses) The 1981[9] paper states that the Factor Method is "recommended to provide the most accurate conversion factor". In that paper, Morr calculates an average NCF of 6.77 and 5.93 for 4 different soy protein preparations analyzed using the Factor Method and Kjeldahl Methods, respectively; calculations for 4 soy proteins whose compositions had been published previously averaged 6.58 In the 1982 paper cited in the IDF report[18], Morr *uses the same amino acid compositional data* he derived in the 1981[9] paper for 2 soy protein preparations, but then "computes" the asparagine and glutamine contents according to the method of Tkachuk 1966[22], 1969[2]; meaning that the content of ammonia was used to *derive* the values for asparagine and glutamine based on the assumption that only these amino acids give rise to the ammonia; the total mole content of ammonia is subtracted from the total moles of asparagine and glutamine to *derive* the value of the carboxylic acid forms of these amino acids which are assumed to be in equal proportion Thus, values for asparagine and glutamine are not consistent with currently known relative proportions of glutamine and asparagine in soy protein

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
Boisen S, Bech-Andersen S and Eggum BO (1987) Acta Agric Scan 37, 299-304[26]	Soy Meal Soy Meal Soy Meal	6.30 (No Amides) 5.65 (With Amides) 5.49	15.87 17.7 18.21	NCFs calculated by even a single research group for a single sample can vary significantly (5.49 to 6.30 for soy meal) and 3 different factors are quoted in this report Note that the IDF report[18] cites this same paper to support a conversion factor range of 6.34 to 6.38 for milk and milk products; this citation provides three different skim milk powder conversion factors: 5.75, 6.13 (corrected for amides) and 6.9; ***The first two factors clearly are not in line with supporting a 6.34-6.38 conversion factor for milk and again demonstrate the problem with consistency in calculation and potential application of different NCFs*** As for the three NCFs provided for soy, 6.3 was calculated based on amino acid composition and protein nitrogen, 5.65 was calculated based on indirect and inaccurate estimates of amidation (measures of ammonia release after acid hydrolysis and *the assumption that all of the ammonia came from asparagine and glutamine*) and 5.49 was calculated based on amino acid nitrogen over total nitrogen, which always gives the lowest value (e.g. 5.75 for skim milk powder using this method)
Mosse J (1990) J Agric Food Chem 38, 18-24[27]	Soy (Glycine max) Soy (Glycine max)	5.38-5.67 5.76	18.18 17.36	The objective of this paper was "to show that in the absence of perfectly accurate values of the conversion factor, it is still possible to accurately determine its upper and lower limits" Mosse questions Jones ,1941 paper[6] "...so that the questionable values he suggested remain still widespread today, in spite of various improvements successively made by Heathcote (1950), Kutscher and Langnau (1965), Tkachuk (1966a,b, 1969, 1977), Tkachuk and Irvine (1969), Ewart (1967), Holt and Sosulski (1979), Sosulski and Holt (1980) and Morr (1981, 1982)". Mosse provides a detailed mathematical approach to determining NCFs (3 possible values k_A k_P and k, depending on calculation method) for 10 cereals and 6 legumes/ oilseeds and shows that the conversion factors that he calculates based on residual amino acids weights change as the nitrogen contents of the samples increased (not always in the same direction depending on the sample type) *providing more evidence for the difficulty in calculating and assuring that analysts use appropriate accurate nitrogen to protein conversion factors* Mosse also pointed out that other researchers have provided NCFs that were in error if they omitted to correct for the amide nitrogen values (coming from asparagine and glutamine); however, his corrections (calculations for k_A) were based on measures of ammonia release after acid hydrolysis and *were based on the assumption that all of the ammonia came from these 2 amino acids only* Mosse's earlier paper (Mosse, 1985 J Cereal Sci 3: 115-130[28]) in wheat cites Tkachuk, 1969[21] as being the only published literature to indicate that all NH3 comes from Gln and Asn alone; latter study also only done in wheat Despite Mosse's claim in current paper that "...the AA compositions used here probably represent the most complete analyses of the total proteins of cultivated seeds" *no amino acid data are provided in the*

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
				paper, appendices or supplemental data, so the reader is not able to verify the calculations made in the paper Amino acid data for non-soy proteins are available (other published papers), *but the data used for soy protein in this paper are "unpublished" and unavailable to view*
Sosulski FW and Holt NW (1980) Can J Plant Sci 60: 1327-1331[29]	Soybean	5.58		In this paper only NCFs for grain legumes were calculated exactly as per Tkachuk, 1969[19] using amino acid analyses; therefore one would expect similar values to those Tkachuk reported It should be noted that using the SAME METHODS, Sosulski and Imafidon (Sosulski and Imafidon, 1990 J Ag Food Chem 38: 1351-1356[30]) reported NCFs of 6.02 to 6.15 for dairy products and 5.61 to 5.93 for egg, meat and fish products
Marriotti F et al. (2008) Crit Rev Food Sci Nutr 48: 177-184[16]	Soybean	5.5		This paper is a review of the issues in calculating NCFs and argues that an NCF of 6.25 is incorrect for **all** major human dietary protein Authors admit addressing this issue has been avoided “because scientists fear opening the Pandora’s box” Marriotti et al point out the flaws with the Jones factors (Jones, 1941[6]) were due to assumptions made and the technology available in 1941 and that amino acid analyses are the preferred method to calculate NCFs, when other additional factors are also taken into account (e.g. non-protein nitrogen). With regard to concerns that amino acid measures have an inherent increased variability compared with measures of nitrogen, Marriotti, et al. point out that the variability of amino acid measures would not significantly impact NCF measures (calculated CV of 2%) and that improvements in amino acid analyses are occurring An interesting point raised by Marriotti, et al. that warrants consideration, is that for proteins with a lower NCF than 6.25, measures of protein content decrease WHILE THE CHEMICAL SCORE (PROTEIN QUALITY) increases (compared to proteins with higher NCFs); example calculations show that more amino acids to meet nutritional requirements are provided in less protein for the protein with lower NCF; this can be avoided if the amino acid requirements are also adjusted for the same NCF
Sriperm N et al. (2011) J Sci Food Agric 91: 1182-1186[31]	Soy meal	5.64		The purpose of this paper was to get to specific NCFs for feedstuffs “to minimize the feeding of excess nitrogen (N) and to reduce N pollution”. Calculations were based on the methods reported by Mosse, 1990[26] so not surprising that soy meal NCF was similar to that of Mosse Interestingly, if the purpose of the paper was to get to specific NCFs to reduce feeding excess N, then one must consider how this information will be used; if the currently used NCF of 6.25 for soy meal in

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
				feed is reduced to 5.64, does the feed formulator add more soy meal to get to the required protein levels and potentially harm the environment by increasing the excreted N in feces? OR should all the existing requirements be lowered in view of the fact that all protein content in feedstuffs, previously based on a NCF of 6.25, should be now considered lower by 5.64/6.25 (reduction of 10%)?If the latter, then there would be NO CHANGE to actual formulations *per se* only a paper exercise to change the nutritional composition for protein.
Maubois J-L and Lorient D (2016) Dairy Sci and Technol 96, 15-25[19]	Soy (Glycine max)	5.61-5.79		This paper, published in a journal devoted to dairy research, is a review that attempts to provide a scientific basis for the nitrogen to protein conversion factors of 6.38 for cow milk protein and 5.71 for soy protein but does not provide primary data to support these NCFs The authors point out the difficulty in obtaining accurate or 'true' nitrogen to protein conversion (NCF) factors; they point out that "…scientists have turned to determining the NCF from the amino acid composition" Interestingly these authors consider low molecular weight peptides and free amino acids as non-protein nitrogen (NPN) but in an earlier paper Mariotti, et al.[16] indicate that there are different objectives when using a NCF and for nutritional considerations all amino acids should be considered in the NCF; this further points out the controversies that arise when using NCFs in general Maubois and Lorient propose that the amino acid sequence of proteins or primary structure of proteins be used to calculate the NCF; this requires a thorough knowledge of the primary structure of proteins which is NOT available for most proteins, but is available for milk proteins; while the major soy protein sequences are known, the overall number of proteins contributing to total protein from soybean[32] is higher than that of milk protein[33] ; therefore it is unlikely that this method would offer any advantages as the relative amounts of the different proteins would need to be known with some certainty and assumed not to change with different lots of protein to develop an accurate NCF This paper cites Utsumi, 1992[34] as being the source of the sequence data on which the calculations of the soy proteins □-conglycinin and glycinin NCF shown in Table 3 are based; Utsumi[33] does not provide direct sequence data but cites other papers, so it is not clear how the NCFs shown in Table 3 were calculated; if one uses the amino acid compositions of □-conglycinin[35] and glycinin[36] to calculate the NCF (using residual weights of the amino acids and weight of nitrogen) for these subunits one obtains 6.31 and 6.36, respectively Similarly when Maubois and Lorient attempt to calculate the soy protein NCF based on relative ratios of □□conglycinin (7S) to glycinin (11S) in soy protein , they do not provide a clear scientific explanation as to how the NCFs are calculated but refer to publications of soy hemagglutinin and its glycosylation; how this impacts the NCF for soy protein is unclear Maubois and Lorient also have a section in the paper on "Processing and anti-nutritional factors" which

Citation	Product Name/ Class	NCF Proposed	%N in Protein	Comments
				are not related to the topic of nitrogen to protein conversion factors; this section is simply added to discount soy protein as a high quality protein for infant formulas and cites very outdated publications and information With regards to suitability for infant formula, the authors attempt to make a case that soy protein is not suitable for infant formula; their unsubstantiated arguments are meaningless in view of the data emerging from the laboratory of Dr. Tom Badger and his Beginnings Study which show that soy protein based formulas promote normal growth and development comparable to cow milk based formulas[37-39] Authors also claim that proposal to use 6.25 NCF for soy protein is unacceptable because it forgets the enormous work conducted over the past 50 years; same can be said for the Jones' factor of 5.71 for soy protein which is still quoted for more than 50 years despite it being based on a faulty logic

Human nutrition research, however, continues to demonstrate that soy is a high-quality protein that supports growth and maintenance when consumed as a sole source protein and 6.25 is used to calculate the protein content of diets.

Table 2. Human Nutrition Studies Assessing Impact of Dietary Soy Protein on Health Outcomes

Citation	Product Name/ Class	NCF used for soy protein	Type of Study & Subjects	Comments
Rand WM et al. (2003) Am J Clin Nutr 77: 109-127[40]	Soy protein	6.25	Meta-Analysis of Nitrogen Balance studies in Adults	This meta-analysis was conducted in response to a request from the FAO/WHO/UNU to assess the protein requirements in healthy adults and tested a variety of animal or plant-based proteins or mixtures of these Protein requirement in adults defined as "the continuing intake of dietary protein that is sufficient to achieve body nitrogen equilibrium (zero balance)…" Despite the known limitations of N balance studies, this method remains the primary approach for determining protein requirement in adults because there is no validate or accepted alternative Studies tested soy protein (7 as sole source and 2 as mixed sources) using an NCF of 6.25 as the basis for determining the quantity of protein intake There were various factors that contributed to the variability in nitrogen balance response due to differences in studies, differences between subjects and differences in subjects day to day; however, there was no significant difference between studies classified as to whether the dietary protein was predominantly from animal, vegetable or mixed-protein sources For the soy studies, the authors concluded: "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)" The authors noted that the major source of dietary protein was found to have an insignificant effect on the median requirement, slope or intercept for nitrogen balance versus nitrogen intake plots One would expect that if the NCF of 6.25 applied to each of the studies led to an overestimation of the actual protein intake, ***then one would expect a lower N balance in the soy protein studies, but this was not observed*** Thus, it can be concluded from these studies that the use of a NCF of 6.25 for soy does not lead to erroneous estimations of protein requirements

Jing H et al. (2010) Early Hum Devel 86: 199-125[37]	Soy protein	6.25	Infant Formula Study to assess effects of breastmilk compared with formula feeding on brain activity in developing infants	Development of brain activity during infancy differs between those who are breastfed compared to with those fed either cow milk or soy protein-based formula, but was generally similar for the formula-fed infants
Andres A et al. (2013) J Pediatr 163: 49-54[38]	Soy protein	6.25	Infant formula study to assess effects of breastmilk compared with formula feeding on body composition and bone mineral content in developing infants	Anthropometric data were similar in soy-formula-fed and cow milk-formula-fed infants; however soy-fed infants were significantly leaner with greater fat-free mass compared with cow-milk formula-fed and breast-fed infants during the first 6 months of life Bone mineral content (BMC) was higher in breast-fed infants compared with cow-milk or soy-formula-fed infants at 3 months, but by age 9 and 12 months BMC was higher in cow-milk and soy-formula-fed infants, with the highest bone mineral accretion occurring in the cow-milk formula fed group

Pivik RT et al. (2013) Intl J Psychophysiol 90: 311-320[39]	Soy protein	6.25	Infant formula study to assess effects of breastmilk compared with formula feeding on cardiovascular development in infants	Although subtle effects of diet and gender were observed, there were no atypical findings with regard to cardiovascular development Differences observed were generally greater between breast-fed and formula-fed groups than between formula-fed infants
Vandenplas Y et al. (2014) Br J Nutr 111: 1340-1360[41]	Soy protein	6.25	Meta-Analysis of Soy Infant formula studies	This is a meta-analysis that reviews the safety of soy infant formula in relation to anthropometric growth, bone health (bone mineral content), immunity, cognition and reproductive and endocrine functions using studies published from 1909 to 2013 The authors concluded that “the patterns of growth, bone health and metabolic, reproductive, endocrine, immune and neurological functions (for soy-based infant formula) are similar to those observed in children fed cow milk-based formula and human milk”

The studies summarized in the Table above indicate that the intake of soy protein, when based on a NCF of 6.25, resulted in similar nitrogen balance in adults and similar growth and development of infants when compared to animal and dairy protein. It is worthy to consider how these results may be interpreted should the NCF of soy protein be changed from 6.25 to 5.71. It could then be considered retrospectively, that 9% less soy protein resulted in similar nitrogen balance and similar infant growth characteristics to that observed with milk protein. Another consideration may be that changing the NCF for soy protein to 5.71 would require reformulating the infant formula to contain more soy protein by weight to meet the infant formula protein requirements. However, that could meet with considerable resistance, since there is a growing body of data that suggest that high dietary protein intakes in infancy and in growing children can induce adverse effects on the risk of obesity and associated diseases[42]. In a multicenter European study, over a thousand healthy term infants were randomly assigned to receive cow milk-based formulas and follow-on formulas with lower (1.77 and 2.2 g protein/100 kcal, respectively) or higher (2.9 and 4.4 g protein/100 kcal, respectively) protein levels[43]. At 2 years of age, the adjusted *z* score for weight-for-length was found to be 0.20 greater (P = 0.005) in the higher- than in the lower-protein formula group[43] and in a follow up of these children at 6 years of age, the high protein group had a significantly higher BMI (by 0.51, P = 0.009) compared to the low protein group [44]. The study investigators also demonstrated that long-term mental performance of children on the low protein intervention was unimpaired compared to the high protein intervention[44] allaying any concerns that reducing protein intake in infancy would have led to any adverse developmental effects. This and other studies then indicate that lowering protein intake in infants, rather than raising protein intake levels, would be associated with a reduced rate of obesity.

Use of the 5.71 factor instead of 6.25 in the calculation of protein content for soy-based follow-up formula could result in excessive protein intake. If grams of protein for a follow-up formula are calculated using a 5.71 nitrogen to protein conversion factor are compared to what the gram amount would be using a 6.25 conversion factor, the protein range would actually be 3.28 – 6.01 g per 100 kcal of FUF (assumes 9.2% reduction in protein content with use of 5.71 vs 6.25), instead of the 3 – 5.5 g/100 kcal range that is listed in the Codex FUF Standard[46].

Table 3. Follow Up Formula Calculations: Protein content using 5.71 vs. 6.25

N Conversion Factor	**3 g protein/100 kcal**	**5.5 g protein/100 kcal**
5.71 (6.25)	3 g (3.28 g)	5.5 g (6.01 g)

With regard to adults, Heidelbaugh ND et al.[47] showed that variations in calculating the protein content of menus or diets using different NCFs derived by different methods, minimally affect the values obtained for total protein contents, since any errors resulting from using 6.25 or specific NCF factors (e.g. Jones' factors) tend to be randomly distributed among any variety of foods when an overall menu containing healthy foods is analyzed. Heidelbaugh ND et al. (1975)[47]demonstrated that the protein content of menus designed for Skylab astronauts, which consisted of 68 different foods, differed by less than 3% when calculated using a NCF of 6.25, using Jones' factors or using derived NCFs based on amino acid composition of the foods. Therefore, it can be said for adult diets which contain a variety of healthy foods, there is no need, based on nutritional considerations, for specific NCFs to calculate protein content for individual foods.

IV. Scientific/Analytical Methodological Environment

Analytical Methods Support a 6.25 Conversion Factor

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. It should be noted that Wolf, et al.[48] reported on the nitrogen content of soybean protein and several fractions of these proteins along with purified proteins. These preparations contained from 16.2 to 16.51% nitrogen[48]. Wolf, et al.[48] reported that a cold insoluble fraction contained 17.46% nitrogen which was probably very similar to the fraction reported by Osborne and Campbell[7].

Application of the 6.25 nitrogen conversion factor to measure soy protein analyzed by Kjeldahl, modified Kjeldahl, and combustion methods is widely recognized by international organizations, such as Codex Alimentarius and FAO[4,5], and technical associations, such as the American Oil Chemists Society (AOCS),

AOAC International (AOAC), AACC International (AACC), and the International Organization for Standardization (ISO).

The Codex Standard 234-1999 "Recommended Methods of Analysis and Sampling" (as amended by the 30th Session of the Commission, 2007)[4] lists AOAC 955.04D method that recognizes 6.25 for soy protein, as the recommended protein measurement method for soy and vegetable protein products. Furthermore, Codex Standard 234-1999[4] specifically states the 6.25 conversion factor should be applied to nitrogen values for soy and vegetable protein products obtained using AOAC 955.04D.

AOCS, AOAC, AACC, and ISO analytical methods are widely recognized by regulatory agencies in enforcement of national regulations, as well as by university and government researchers. The current protein analytical methods approved by membership consensus in these technical associations list 6.25 as the nitrogen conversion factor for soy protein (Table 4).

Table 4. Official AOCS, AOAC, AACC, and ISO Soy Protein Analytical Methods

Current Protein Analytical Method	Recommended Nitrogen Conversion Factor
AOCS Ac 4-91[49](Revised 2011)	6.25
AOCS Ba 4d-90[50] (Revised 2011)	6.25
AOCS Ba 4e-93[51] (Revised 2011)	6.25
AOCS Ba 4f-00[52] (Revised 2011	6.25
AOCS Ba 4a-38[53] (Revised 2011)	6.25
AOCS Ba 10-65[54] (Reprinted 2009)	6.25
AOCS Ba 10a-05[55] (Reprinted 2009)	6.25
AOAC 992.23[56] (Revised 2005)	6.25
AACC 46-10.01[57] (Reapproval 1999)	6.25
AACC 46-11.02[58](Reapproval 1999)	6.25
AACC 46-16.01[59] (Reapproval 1999)	6.25
AACC 46-30.01[60] (Reapproval 1999)	6.25
ISO 16634-1:2008[61]	6.25

Newer Protein Analysis Methods Provide More Accurate Protein Data and Prove 5.71 Conversion Factor for Soy is Incorrect

The 5.71 nitrogen conversion factor for soy protein is based on analytical data generated by D.B. Jones, Principal Chemist of the United States Department of Agriculture (USDA) in a Circular (1931, slightly revised 1941)[6]. In this Circular[6], 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. He also reported a wide variation in the nitrogen content across these protein sources. Jones justified the 5.71 factor for soybeans by stating 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).

This 5.71 conversion factor for soy protein, based on Jones' logic, is false.

Research[8, 10, 11] has shown, however, that there can be wide variations in the levels of the major proteins in soybeans, glycinin and ß-conglycinin, which could result in widely different nitrogen conversion factors if Jones' logic were carried out. Murphy and Resurreccion (1984)[8] found glycinin/ß-conglycinin ratios varied significantly, depending on the soybean variety and differences in seasonal growing conditions. Roberts and Briggs (1965)[10] and Koshiyama (1968)[11] found that soy proteins typically consist of about 35% ß-conglycinin and contain between 15.5%[9] - 15.9%[10] nitrogen, respectively, translating to a conversion factor of 6.45 – 6.29.

In recognition of the inconsistencies and inaccuracies inherent in analytical methods that measure protein indirectly through nitrogen content, other methods for measuring protein have been developed. In December of 2002, FAO convened the "Technical Workshop on Food Energy: Methods of Analysis and Conversion Factors". Outcomes of this workshop were published in FAO Food and Nutrition Paper 77[5]. One of the significant outcomes of this workshop was the recommendation by the expert panel for a superior and more accurate method using the sum of the anhydrous amino acids to measure protein. That is:

To measure protein as the sum of individual anhydrous amino acids, rather than the measurement of nitrogen by the Kjeldahl and other indirect methods.

Further, the workshop participants recommended that food composition tables should express protein content by the sum of anhydrous amino acids whenever possible, so these data may be used globally[5].

Using this recommended method, analytical product data supports a 6.25 nitrogen conversion factor as discussed below.

V. Analytical Product Data Using FAO's 2003 Recommendation

Analytical Product Data Supporting 6.25 Nitrogen Conversion Factor

The FAO Food and Nutrition Paper 77[5] recommended protein measurement by amino acid analyses; if one applies this method to calculating the nitrogen conversion factors for defatted soybean meal, soy protein concentrate, and isolated soy protein one obtains values that range from 6.24 – 6.37 (Tables 5-7). The amino acid content of various soy ingredients produced from 1993-2007 were measured using a modification of the method described in Morr, 1981[9]. The anhydrous amino acid content was calculated as the amino acid molecular mass minus the molecular weight of water.

In addition, application of the FAO method to isolated soy protein amino acid data from 1982, isolated soy protein data currently available on the USDA National Nutrient Database for Standard Reference[29,30], and to amino acid data independently published in the scientific literature by Morr, 1981[9] yield a 6.30-6.31 conversion factor for soy protein. Application of the FAO method to amino acid values to commonly consumed foods, like soymilk[62] and tofu[63], published in the USDA National Nutrient Database for Standard Reference yields a 6.30 conversion factor.

The nitrogen conversion factors calculated from a fifteen year span of amino acid data demonstrate an overall average value of 6.33 (Tables 5-7). With the exception of one data point at 6.24 for one lot of defatted soy meal, the remaining nitrogen conversion factor values vary from 6.29 – 6.37. It is well recognized by experts in the field that plant products exhibit natural year-to-year differences and product-to-product differences, which are to be expected due to different growing conditions and variations in manufacturing processes. The data for isolated soy protein ingredients presented in this document demonstrate stability of the protein nitrogen conversion factor over a 15 year period of time (Tables 5-7).

Amino acid analyses were performed on 55 soy protein samples (flakes and flour, isolated soy protein (ISP) or soy protein concentrates (SPC) according to conventional methods[64]. Samples were subject to acid hydrolysis at 110^{o}C for 24 hours and the amino acids were separated by ion exchange chromatography and detected with ninhydrin. Each amino acid was quantitated against a standard known concentration for aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. Methionine and cysteine were also quantitated after performic acid oxidation and tryptophan was quantitated after sodium hydroxide hydrolysis[64]. Values for amino acid weights were used to calculate a nitrogen conversion factor as described by Morr[9].

The data in Tables 5-7 are based on analytical data from daily production samples analyzed by a single independent laboratory and show a nitrogen to protein ratio that is greater than the value, 6.25. Amino acid data used to calculate values for NCF of Isolated Soy Protein (2004-2007), soy protein concentrate, and soy flakes shown in Tables 5, 6, and 7, respectively, can be found in the Appendix as Tables 11-13.

Very importantly, it is noteworthy that these data are much more consistent with a nitrogen conversion factor of 6.25 than 5.71.

Table 5. 1993-2007 Isolated Soy Protein Industry Data*, **

Year	N Conversion Factor
1993	6.31
1994	6.33
1994	6.31
1995	6.33
1995	6.32
1997	6.35
1997	6.34
1998	6.36
1998	6.36
2002	6.33
2002	6.33

2002	6.32
2002	6.33
2003	6.34
2003	6.35
2004	6.35
2004	6.33
2004	6.36
2004	6.34
2004	6.34
2005	6.36
2005	6.35
2005	6.37
2005	6.34
2006	6.31
2006	6.35
2006	6.36
2006	6.34
2006	6.36
2006	6.33
2006	6.36
2007	6.31
2007	6.30
2007	6.31
2007	6.32
Mean	6.34
Standard Deviation	0.02

*Analytical method adapted from Morr, 1981[9]

** NPAL Analytical Laboratories (St. Louis, MO, USA)

Table 6. 2004-2007 DuPont Soy Protein Concentrate Product Data*, **

Year	N Conversion Factor
2004	6.31
2004	6.29
2004	6.34
2004	6.32
2005	6.35
2005	6.37
2005	6.32
2006	6.32
2006	6.32
2007	6.29

Mean 6.32

Standard Deviation 0.03

*Analytical method adapted from Morr, 1981[9]

** NPAL Analytical Laboratories (St. Louis, MO, USA)

Table 7. 2005-2007 DuPont Soy Flake & Flour Product Data*, **

Year	N Conversion Factor
2005	6.30
2005	6.31
2005	6.31
2004	6.34
2005	6.31
2005	6.32
2005	6.31
2005	6.24
2006	6.31
2007	6.29

Mean 6.30

Standard Deviation 0.03

*Analytical method adapted from Morr, 1981[9]

** NPAL Analytical Laboratories (St. Louis, MO, USA)

In order to perform amino acid analysis on intact protein, it is necessary to release the constituent amino acids using hydrolysis. This is most commonly done via acid hydrolysis in 6N HCl over a period of time. Acid hydrolysis results in the conversion of amidated amino acids (glutamine and asparagine) to their acidic counterparts (aspartate and glutamate). Thus, during analysis, glutamine and glutamate are quantitated together, as are asparagine and aspartate. Since the amidated amino acids contain two nitrogen molecules and the acidic forms one, one cannot accurately calculate a NCF using amino acid analysis data alone, since one cannot accurately determine amidated amino acid content.

There is currently no method for direct quantitation of both glutamine and asparagine from protein. In 1966, Tkachuk[22] described two separate methods for estimating the amounts of amidated amino acids in protein samples. In the first method, amide ammonia released during hydrolysis is measured at several time points, then extrapolated to zero to estimate the concentration of amidated amino acids present in the starting sample. This method assumes linearity throughout the hydrolysis process, and is an extrapolation from only three time points. In 1982, Morr[25] published a research note in which he recalculated nitrogen conversion factors for soy products using the ammonia estimation method of Tkachuk[20]. In this note, Morr reduced the factors to 5.66-5.79 for four soy products based on an estimation of the amount of glutamine and asparagine present in each product[25]. Given that Tkachuk's method[20] is based on estimation of amide content in wheat, one cannot conclude that those factors calculated by Morr, 1982[25] are accurate.

In the second method referenced in Tkachuk, 1966[22], he attempts to determine amidated amino acid concentrations using three separate hydrolytic enzymes prepared in his laboratory using published methods. It should be noted that any side activities in these preparations had not been measured; it was assumed that no asparagine or glutamine deamidase activity was present that would lead to inaccurate results. In order to obtain concentrations for glutamine and asparagine, Tkachuk[22] performed both enzymatic and acid hydrolyses on samples, separated the resultant amino acids by chromatography, then compared the two chromatograms to determine differences. It should be noted that glutamine and asparagine were presumed by Tkachuk[22] to co-elute with serine (based on retention times measured using pure standards). Thus, he could only estimate the amount of each by measuring differences in the serine peak between acid hydrolyzed and enzymatically hydrolyzed samples. Direct measurement of asparagine and glutamine released by this method was not possible. In addition, amino acid recoveries using the enzymatic method were poor, reaching only approx. 80% compared to >90% for the acid hydrolysis method. Thus, although valiant, Tkachuk's second method[22] can only be viewed as means of approximating the levels of asparagine and glutamine present in intact proteins.

Recently, a method was published using derivatization with [bis(trifluoroacetoxy)iodo]benzene (BTI) to measure glutamine levels in intact proteins[65]. Under the appropriate conditions, this reagent converts bound glutamine to acid-stable L-2,4-diaminobutyric acid (DABA). Thus, one can quantitate glutamine by measuring the DABA released following acid hydrolysis. BTI also converts asparagine to L-2,3-diaminopropionic acid (DAPA). However, Kuhn, et al.[65] have reported poor recovery of DAPA upon hydrolysis, so were unable to use this method for asparagine quantitation.

In conclusion, use of the Morr Factor method[25] to determine NCFs from anhydrous amino acid data can only approximate the factor, because it is not currently possible to measure asparagine and glutamine concentrations using direct methods. Therefore, use of NCFs derived from amino acid analysis data can only be viewed as estimates, until such time when validated, quantitative methods for determination of all amino acids present in a given sample are developed.

Use of the 5.71 Conversion Factor Conflicts with Mass Balance Calculations

As part of a quality assurance program, soy protein ingredient manufacturers generally analyze protein, moisture, fat, and ash for each lot of product. These proximates are all measured by direct analysis. Carbohydrates are not directly analyzed. Carbohydrate values are calculated by difference[4]: 100 minus the sum of protein, moisture, fat, and ash. Therefore, proximates must always add up to 100%. Isolated soy protein typically contains <1% carbohydrate, as determined by calculation[4]. Typical proximate values (on dry matter basis) for isolated soy protein using 6.25 as the conversion factor generate proximate data that can be supported by direct analysis (Table 8). Typical values for isolated soy protein using 5.71 as the conversion factor, however, generate proximate data that cannot be supported by direct analysis (Table 9). Use of the 5.71 factor results in 8% “missing mass”. This 8% fraction cannot be properly classified as a nutrient by analytical methods, as the proximate values do not add up to 100% .

Table 8. 6.25 Factor: Typical Macronutrient Data for Isolated Soy Protein

Macronutrient	**Typical Value**
Protein (dry matter basis)	91%
Fat	4%
Ash	4%
Carbohydrate	1%

Table 9. 5.71 Factor: Typical Macronutrient Data for Isolated Soy Protein

Macronutrient	**Typical Value**
Protein (dry matter basis)	83%
Fat	4%
Ash	4%
Carbohydrate	1%
Missing Mass	**8%**

VI. Regulatory Environment

International Product Standards and Nutrition Labeling Recommendations and Regulations

Use of the 6.25 nitrogen conversion factor for soy protein is widely recognized as the appropriate method to determine compliance with product standards and nutritional labeling regulations by international organizations, such as Codex Alimentarius, and government regulatory agencies in India, Japan, Korea, the European Union, the United States, Argentina, and Brazil (Table 10). Although an exhaustive list of regulations from around the globe is not provided in this document, the data provided represent the nutrition labeling regulations for a significant portion of the world’s population[66].

The 2007 FAO/WHO Compendium of Codex Standards for Cereals, Pulses, Legumes, and Vegetable Proteins[67] and current Codex standards specifically state the 6.25 conversion factor should be applied to calculate protein values for soy and vegetable protein products. Namely:

- 175-1989 ”Codex General Standard for Soy Protein Products”[1]

- 174-1989 “Codex General Standard for Vegetable Protein Products (VPP)[2]
- CAC/GL 2-1985 “Guidelines on Nutrition Labelling” (as amended by the 29th Session of the Commission, 2006)[3]

Codex Standard 175-1989[1] is widely accepted and followed by the isolated soy protein industry. Additionally, the 90% minimum protein level stated in Codex Standard 175-1989[1] serves as an important product standard to help identify high value isolated soy protein.

The nutrition labeling regulations of many major trading blocs list the 6.25 nitrogen conversion factor. For example, Argentina[68], Brazil[69], China[70], the European Union[71], India[72], Japan[73], Korea[74], and the United States[75] all require a 6.25 nitrogen conversion factor for soy protein ingredients. In addition, these nations recognize the Codex General Standard for Soy Protein Products STAN 175-1989[1], which requires a minimum 90% protein content.

Table 10. Current Soy Protein Conversion Factors from Around the Globe

Organization/Country/Region	Standard/Regulation	N Conversion Factor
Codex	Codex General Standard for Soy Protein Products STAN 175-1989[1]	6.25
Codex	Codex General Standard for Vegetable Protein Products (VPP) STAN 174-1989[2]	6.25
Codex	Guidelines on Nutrition Labelling CAC/GL 2-1985[3]	6.25
Argentina	Laws for the Labeling and Advertising of Food: Resolution in Conjunction with SPRyRS 149/2005 y SAGPyA 683/2005[68]	6.25
Brazil	Brazil National Health Surveillance Agency (ANVISA). Resolution – RDC No. 268, September 22, 2005[69]	6.25
China	China Ministry of Health “GB5009.5 Determination of Protein in Food”[70]	6.25
European Union	Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers[71]	6.25
India	Lab. Manual 3, Manual of Methods of Analysis of Foods, Cereal and Cereal Products, Directorate General of Health Services Ministry of Health and Family Welfare, Government of India[72] 2005[71]	6.25
Japan	Japanese Agricultural Standard for Vegetable Protein and Seasoned Vegetable Protein[73]	6.25
Korea	Nitrogen Conversion Factors for Protein Calculation, Korea Food Code[74]	6.25
United States	Title 21 Code of Federal Regulations Part 101.9[75]	6.25

VVII. Implications of the Change from 6.25 to 5.71 Nitrogen Conversion Factor

Changing the nitrogen conversion factor for soy protein from the widely accepted 6.25 to 5.71 could have significant implications:

- Elimination of isolated soy protein as a food ingredient from the marketplace as it will be impossible to meet the product standard 90% protein minimum using 5.71 factor
- Significant costs to food manufacturers due to expensive label changes
    - "Isolated soy protein" would have to be removed from product ingredient lists
    - Changes to protein nutrition labeling
    - Potential requirement for product formula changes
- Confusion for food manufacturers seeking to make products containing isolated soy protein
- Confusion for consumers seeking products containing isolated soy protein
- Impacts on presentation and interpretation of data from nutritional research for both scientific and lay audiences (which use 6.25 for protein calculations)
- Significant cost increases for animal production facilities using soy as source of protein in feed rations
- Trade and product labeling logistical difficulties presented with multiple nitrogen conversion factors for various protein sources

Current isolated soy protein production methods generate product with a typical protein range of 90-92%, using 6.25 as the conversion factor. Occasionally, protein levels can reach 93-94%. Use of the 5.71 conversion factor for soy protein would artificially eliminate the isolated soy protein category, as protein levels will not reach the 90% minimum for the product standard. Product that is currently labeled as "isolated soy protein" would now be identified as "soy protein concentrate" (Codex STAN 175-1989 defines protein levels for soy protein concentrate as <90%, but ≥65%[1]). When 5.71 is applied, typical protein values would change to 82-84%, with occasional levels of 85-85.9%. Resulting replacement of the terminology "isolated soy protein" with "soy protein concentrate" in the ingredient list as a result of the use of a 5.71 conversion factor would require costly label changes for any product formula currently containing isolated soy protein.

In addition, products containing soy protein imported from countries utilizing the 6.25 conversion factor would require significant label changes. These significant label changes could generate confusion amongst consumers seeking products made with isolated soy protein, as well as products with specific protein levels. Furthermore, the use of a 5.71 factor for soy protein and the indirect measurement of protein via nitrogen content could inadvertently encourage adulteration of protein containing soy foods with substances that deliver nitrogen, as food processors may wish, for example, to continue to produce product with similar nutritional profiles and similar product standards of identity.

Soy protein has long been recognized for its beneficial health effects. As a result, soy protein has been extensively used in pre-clinical and clinical nutrition research. An important aspect of reporting data from nutrition studies for publication in international scientific research journals is the quantification of dietary protein intake. If the 5.71 factor is utilized to assess dietary soy protein intake while other countries use 6.25, the data may reflect artificially, yet significantly lower protein intakes in studies that utilize soy protein and the incorrect 5.71 factor. These artificially lower protein intakes in studies could conflict with soy research data generated from dietary intervention trials from other parts of the globe, making comparability of results across studies a challenge.

Animal production facilities that utilize soy as a significant protein source will face increased costs for feed if current feeding rates and amounts were maintained, due to the fact that measurement of protein levels in soy using the 5.71 factor will result in feed with 8.6% lower protein than levels calculated using the 6.25 NCF. Increasing the soy protein in animal feeds (if the NCF was reduced to 5.71) will also most certainly increase the nitrogen released in the feces of the monogastric animals which is harmful to the environment as pointed out by Mosse, et al.[27].

Finally, if the 5.71 conversion factor were to be applied to soy protein based on the 1931 research conducted by Jones[6], it should follow that the NCFs should be revisited for ALL major food proteins. Jones cited several NCFs for various proteins. As is the case with soy protein, it is likely that several of the NCFs reported by Jones are potentially incorrect due to the lack of sophisticated analytical techniques in 1931 compared to more recent technological advancements, as has already been pointed out by several researchers including Mosse, et al[27]. Determination of unique NCFs for all proteins from different sources that may be found in the food supply will be extremely laborious and will require consensus on a single

method of calculating this NCF. Even if this is realized, implementing the agreed upon NCF for all proteins globally will be most difficult. It would appear more prudent to spend resources to develop methods that are based on amino acids themselves (as the nutritionally relevant moiety of the protein) rather than continue the decades long debate as to which NCFs are appropriate for different proteins.

VIII. Conclusions

In conclusion, this position document has carefully documented both regulatory and scientific support for the validity of 6.25 as the soy protein NCF. Additionally, as recommended by the FAO in 2003 and in the interests of continued advancement of analytical testing technology and food safety and quality, we also respectfully submit for consideration the measurement of protein via the sum of anhydrous amino acids, rather than the indirect measurement of protein obtained from the Kjeldahl method. Recent efforts to improve the measures of protein quality assessment are based on amino acid analyses, so it is reasonable to expect that such methods will be standardized and more readily accessible globally. In addition, credible and valid analytical data on a variety of ingredients has been included that further support 6.25 as the soy NCF. We therefore, respectfully request the continued use of the 6.25 NCF for the measurement of protein in soy products. Harmonization of nutritional labeling and product standards, across professional organization and governments, is best served by continuing the 6.25 NCF for soy protein.

IX. References

1. Codex Alimentarius STAN 175-1989 Codex general standard for soy protein products.

2. Codex Alimentarius STAN 174-1989 Codex general standard for vegetable protein products (VPP).

3. Codex Alimentarius CAC/GL 2-1985 Guidelines on nutrition labelling.

4. Codex Alimentarius STAN 234-1999 Recommended Methods of Analysis and Sampling.

5. Food and Agriculture Organization of the United Nations (2003) Food energy – methods of analysis and conversion factors. Report of a technical workshop Rome, 3-6 December 2002.

6. 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.

7. Osborne, TB and Campbell, GF (1898) Proteids of the soybean (Glycine hispida). Journal of the American Chemical Society 20: 419-428.

8. 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.

9. Morr, CV (1981) Nitrogen Conversion Factors for Several Soybean Protein Products. Journal of Food Science 46: 1362-67.

10. Roberts, RC and Briggs, DR (1965) Isolation and Characterization of the 7S Component of Soybean Globulins. Cereal Chem 42:71.

11. Koshiyama, I (1968) Chromatographic and sedimentation behavior of a purified 7S protein in soybean globulin. Cereal Chem 45:405.

12. Angell AR, Mata L, de Nys R, Paul NA (2015) The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J Applied Phycology 28(1):511-524.

13. Koletzko, B and Shamir, R (2006) Standards for infant formula milk. Brit Med J 332:621-622.

14. Food and Agriculture Organization of the United Nations (2013) Edible insects: future prospects for food and feed security. FAO Forestry Report #171.

15. Ministry of Education, Culture, Sports, Science and Technology – Japan. Standard Tables of Food Composition in Japan - 2015 - (Seventh Revised Version).

http://www.mext.go.jp/english/science_technology/1347490.htm

Accessed January 28, 2016

16. Marriotti F, Tome D, Mirand PP (2008) Converting nitrogen into protein--beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr 48(2):177-84.

17. Joint FAO/WHO Food Standards Programme (2015) Codex Alimentarius Commission Procedural Manual, Twenty-fourth ed.

18. Comprehensive review of scientific literature pertaining to nitrogen protein conversion factors (2006) Bulletin of the International Dairy Federation 405

19. Maubois, JL and Lorient, D (2016) Dairy protein and soy protein in infant foods nitrogen-to-protein conversion factors. Dairy Sci & Technol 96: 15-25.

20. Tkachuk R (1969) Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem 46: 419-423.

21. Tkachuk, R and Irvine, GN (1969) Amino acid compositions of cereals and oilseed meals. Cereal Chem 46:206-218.

22. Tkachuk, R (1966) Amino acid composition of wheat flours. Cereal Chem 43:207-222.

23. DeRham O (1982) La proportion d'azote dans les proteins et le facteur de calcul proteine/azote. Lebensm. Wiss. Technol 15:226-231.

24. Food and Agriculture Organization of the United Nations (1970) Amino-Acid Content of Foods and Biological Data on Proteins.

25. Morr (1982) Recalculated nitrogen conversion factors for several soybean protein products. J Food Sci 47(5):1751-1752.

26. Boisen S, Bech-Andersen S, Eggum BO (1987) A critical view on the conversion factor 6.25 from total nitrogen to protein. Acta Agric Scand 37(3):299-304.

27. Mosse, 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 Seed Protein Content. Journal of Agricultural Food Chemistry 38: 18-24.

28. Mosse J, Huet JC, Baudet J (1985) The amino acid composition of wheat grain as a function of nitrogen content. J Cereal Sci 3(2):115-130.

29. Sosluski FW and Holt NW (1980) Amino acid composition and nitrogen-to-protein factors for grain legumes. Canadian J Plant Sci 60(4):1327-1331.

30. Sosulski, FW and Imafidon, GI (1990) Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J Agric Food Chem 38 (6):1351–1356.

31. Sriperm N, Pesti GM, Tillman PB (2011) Evaluation of the fixed nitrogen-to-protein (N:P) conversion factor (6.25)versus ingredient specific N:P conversion factors in feedstuffs. J of Sci Food and Agric 91(7):1182–1186.

32. Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature 463 (14):178-183.

33. Farrell HM, et al. (2004) Nomenclature of the proteins of cow's milk – sixth revision. J Dairy Sci 87:1641-1674.

34. Utsumi, S (1992) Plant food protein engineering. In: Kinsella JE (ed) Advances in food and nutrition research, vol 36. Academic, San Diego, pp 89-206.

35. Zheng H-G, Yang X-Q, Ahmad I, Min W, Zhu J-H and Yuan D-B (2009) Soybean □-conglycinin consitutent subunits: Isolation, solubility and amino acid composition. Food Res Intl 42:998-1003.

36. Catsimpoolas N, Kenney JA, Meyer EW and Szuhaj BF (1971) Molecular weight and amino acid composition of glycinin subunits. J Sci Food Agric 22: 448-450.

37. Jing H, Gilchrist JM, Badger TM, Pivik RT (2010) A longitudinal study of differences in electroencephalographic activity among breastfed, milk formula-fed, and soy formula-fed infants during the first year of life. Early Hum Dev 86(2):119-25.

38. Andres A, Casey PH, Cleves MA, Badger TM (2013) Body fat and bone mineral content of infants fed breast milk, cow's milk formula, or soy formula during the first year of life. J Pediatr 163(1):49-54.

39. Pivik RT, Andres A, Tennal KB, Gu Y, Armbya N, Cleves MA, Badger TM (2013) Infant diet, gender and the normative development of vagal tone and heart period during the first two years of life. Int J Psychophysiol 90(3):311-20.

40. Rand WM, Pellett PL, Young VR (2003) Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. Am J Clin Nutr 77(1):109-27.

41. Vandenplas Y, Castrellon PG, Rivas R, Gutiérrez CJ, Garcia LD, Jimenez JE, Anzo A, Hegar B, Alarcon P (2014) Safety of soya-based infant formulas in children. Br J Nutr 111(8):1340-60.

42. Koletzko B, Demmelmair H, Grote V, Prell C, Weber M (2016) High protein intake in young children and increased weight gain and obesity risk. Am J Clin Nutr 103(2):303-4.

43. Koletzko B, von Kries R, Closa R, Escribano J, Scaglioni S, et al. (2009) Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. Am J Clin Nutr 89: 1836-1845.

44. Weber M, Grote V, Closa-Monasterolo R, Escribano J, Langhendries J-P, et al. (2014) Lower protein content in infant formula reduces BMI and obesity risk at school age: follow-up of a randomized trial. Am J clin Nutr 99:1041-1051.

45. Escribano J, et al. (2016) Mental performance in 8-year-old children fed reduced protein content formula during the 1st year of life: safety analysis of a randomised clinical trial. Br J Nutr 22:1-9. doi:10.1017/S0007114515000768

46. Codex Alimentarius CODEX STAN CAC/GL 72-1981 Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants.

47. Heidelbaugh ND, Huber CS, Bednarczyk JF, Smith MC, Rambaut PC, Wheeler HO (1975) Comparison of three methods for calculating protein content of foods [Skylab foods, amino acid analyses]. J Agric Food Chem 23(4):611-13.

48. Wolf, WJ, Sly DA and Kwolek, WF (1966) Carbohydrate content of soybean proteins. Cereal Chemistry 43: 80-94.

49. AOCS Official Method Ac 4-91, Revised 2011. Nitrogen-Ammonia-Protein Modified Kjeldahl Method Titanium Dioxide + Copper Sulfate Catalyst.

50. AOCS Official Method Ba 4d-90, Revised 2011. Nitrogen-amonia-protein Modified Kjeldahl Method Titanium Dioxide + Copper Sulfate Catalyst.

51. AOCS Official Method Ba 4e-93, Revised 2011. Generic Combustion Method for Determination of Crude Protein.

52. AOCS Official Method Ba 4f-00, Revised 2011. Combustion Method for Determination of Crude Protein in Soybean Meal.

53. AOCS Official Method Ba 4a-38, Revised 2011. Nitrogen-amonia-protein Modified Kjeldahl Method.

54. AOCS Official Method Ba 10-65, Reprinted 2009. Protein Dispersibility Index (PDI).

55. AOCS Recommended Practice Ba 10a-05, Reprinted 2009. Protein Dispersibility Index (PDI).

56. AOAC Official Method 992.23, 18th ed, 2005. Crude Protein in Cereal Grains and Oilseeds: Generic Combustion Method; First Action 1992.

57. AACC International Method 46-10.01, Final approval April 13, 1961; Reapproval November 3, 1999. Crude Protein—Improved Kjeldahl Method.

58. AACC International Method 46-11.02, Final approval October 8, 1976; Reapproval November 3, 1999. Crude Protein—Improved Kjeldahl Method, Copper Catalyst Modification.

59. AACC International Method 46-16.01, First approval October 12, 1988; Reapproval November 3, 1999. Crude Protein—Improved Kjeldahl Method, Copper-Titanium Dioxide Catalyst Modification.

60. AACC International Method 46-30.01, Final approval November 8, 1995; Reapproval November 3, 1999. Crude Protein—Combustion Method.

61. ISO 16634-1:2008, Approved November 1, 2008. Food Products: Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content. Part 1: Oilseeds and Animal Feeding Stuffs.

62. USDA National Nutrient Database for Standard Reference, Release 21 (2008) USDA Agricultural Research Service. 100 g Data for Soymilk, Original and Vanilla, Unfortified.

Accessed September 9, 2009.

http://www.nal.usda.gov/fnic/foodcomp/search/

63. USDA National Nutrient Database for Standard Reference, Release 21 (2008) USDA Agricultural Research Service. 100 g Data for Tofu, Firm, Prepared with Calcium Sulfate and Magnesium Chloride (Nigari).

Accessed September 9, 2009.

http://www.nal.usda.gov/fnic/foodcomp/search/

64. Otter, DE (2012) Standardised methods for amino acid analysis of food. Br J Nutr 108: S230-237.

65. Kuhn, KS, et al. (1996) Quantitative Analyses of Glutamine in Peptides and Proteins. J Agric Food Chem 44(7):1808-1811.

66. International Data Base (IDB). United States Census Bureau. Country Rankings.

Accessed September 9, 2009.

http://www.census.gov/ipc/www/idb/

67. Food and Agriculture Organization of the United Nations & World Health Organization (2007) Cereals, Pulses, Legumes and Vegetable Proteins, 1st ed.

68. Argentina Laws for the Labeling and Advertising of Food: Resolution in Conjuction with SPRyRS 149/2005 y SAGPyA 683/2005.

69. Brazil National Health Surveillance Agency (ANVISA). Resolution - RDC No. 268, September 22, 2005.

70. China Ministry of Health "GB5009.5 Determination of Protein in Food".

71. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers.

72. Lab. Manual 3. Manual of Methods of Analysis of Foods, Cereal and Cereal Products. Directorate General of Health Services, Ministry of Health and Family Welfare,

Government of India, 2005.

73. Japanese Agricultural Standard for Vegetable Protein and Seasoned Vegetable Protein, Standard accessed August 28, 2009.

74. Nitrogen Conversion Factors for Protein Calculation, Korea Food Code.

75. Title 21 United States Code of Federal Regulations, Part 101.9.

X. Appendix

Acronyms

AACC	AACC International (previously known as American Association of Cereal Chemists)
AOAC	AOAC International (previously known as Association of Official Analytical Chemists)
AOCS	American Oil Chemists Society
FAO	Food & Agriculture Organization of the United Nations
ISP	Isolated Soy Protein
ISO	International Organization for Standardization
SPC	Soy Protein Concentrate
USDA	United States Department of Agriculture
WHO	The World Health Organization

Table 11: Calculation of Nitrogen Conversion Factors for Soy Protein Isolate from anhydrous amino acid data

	g anhydrous AA residue/100 g sample																	
AA	**2004**	**2004**	**2004**	**2004**	**2005**	**2005**	**2005**	**2005**	**2006**	**2006**	**2006**	**2006**	**2006**	**2006**	**2007**	**2007**	**2007**	**2007**	**Averages**
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	5.50	5.37	5.39	5.36	**5.47**
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**
Arg	6.86	6.80	6.71	6.76	6.75	6.84	6.84	6.74	6.58	6.79	6.72	6.70	6.80	6.72	6.72	6.68	6.57	6.62	**6.73**
Asp	10.26	9.74	9.70	9.84	10.15	10.23	10.19	9.77	9.66	9.97	10.03	10.06	9.82	10.05	9.77	9.62	9.66	9.77	**9.90**
Thr	3.19	3.06	3.05	3.10	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**
Ser	4.34	4.16	4.11	4.23	4.26	4.26	4.22	4.10	4.19	4.16	4.20	4.22	4.18	4.09	4.08	4.03	4.01	4.04	**4.16**
GlutA	18.29	18.10	17.90	18.12	18.41	18.74	18.67	18.21	16.25	18.63	18.17	17.91	19.21	18.38	16.73	16.42	16.45	16.45	**17.84**
Pro	4.36	4.77	4.48	4.40	4.54	4.49	4.50	4.48	4.60	4.58	4.73	4.55	4.64	4.79	4.50	4.50	4.33	4.45	**4.54**
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**
Ala	3.56	3.32	3.36	3.41	3.45	3.40	3.35	3.37	3.40	3.24	3.38	3.39	3.36	3.40	3.34	3.29	3.34	3.37	**3.37**
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**
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	4.10	4.40	4.17	4.20	4.18	4.24	**4.13**
Meth	1.12	1.27	1.23	1.19	1.15	1.11	1.13	1.13	1.16	1.16	1.10	1.09	1.14	1.14	1.11	1.14	1.15	1.14	**1.15**
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**
Leu	7.20	6.77	6.83	6.93	6.94	6.89	6.92	6.75	6.85	6.68	6.97	7.08	6.90	6.88	6.77	6.70	6.75	6.84	**6.87**
Tyr	3.57	3.41	3.35	3.49	3.46	3.41	3.48	3.40	3.46	3.36	3.45	3.51	3.44	3.45	3.43	3.33	3.37	3.37	**3.43**
PhenylA	4.82	4.50	4.46	4.60	4.69	4.66	4.69	4.47	4.45	4.45	4.73	4.81	4.57	4.55	4.51	4.42	4.40	4.53	**4.57**
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**
g protein/100g sample	88.65	86.72	85.69	86.63	87.74	87.96	87.66	85.98	84.09	86.22	87.07	87.89	87.71	87.72	84.71	83.81	83.69	84.43	**86.36**
Total g N /100 g sample	13.96	13.69	13.52	13.65	13.8	13.85	13.77	13.57	13.32	13.58	13.68	13.87	13.79	13.8	13.43	13.3	13.26	13.36	**13.62**
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	6.30	6.31	6.32	**6.34**

Standard Amino Acid Analysis was performed as described in the text (see Section V above). Anhydrous amino acid weights were calculated by subtracting the MW of water (18 Da) from each amino acid, and the resultant weights tallied to determine percent protein content in a 100 gm sample. Total sample nitrogen was determined by tallying the N present in each AA residue based on percent nitrogen values. NCF was determined by dividing protein content by total nitrogen.

Table 12: Calculation of Nitrogen Conversion Factors for Soy Protein Concentrates from anhydrous amino acid data

	g anhydrous AA residue/100 g sample							
AA	**2004**	**2004**	**2004**	**2004**	**2005**	**2005**	**2005**	**Averages**
lys	5.59	5.52	5.58	5.60	5.51	5.54	5.51	**5.55**
Hist	2.14	2.10	2.09	2.10	2.09	2.07	2.11	**2.10**
NH#	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Arg	6.71	6.59	6.56	6.56	6.57	6.67	6.57	**6.61**
Asp	9.87	9.72	9.96	9.81	10.14	10.33	9.80	**9.95**
Thr	3.25	3.18	3.22	3.24	3.25	3.18	3.23	**3.22**
Ser	4.25	4.21	4.28	4.20	4.23	4.32	4.19	**4.24**
GlutA	17.55	17.23	17.88	17.55	17.95	18.75	17.63	**17.79**
Pro	4.41	4.34	4.56	4.43	4.55	4.66	4.39	**4.48**
Glyc	3.15	3.76	3.19	3.13	3.21	3.18	3.12	**3.25**
Ala	3.48	3.42	3.48	3.45	3.49	3.48	3.41	**3.46**
Cyst	1.30	1.30	1.29	1.28	1.19	1.12	1.29	**1.25**
Val	4.02	3.97	4.19	4.11	4.18	4.17	3.96	**4.09**
Meth	1.34	1.30	1.29	1.31	1.25	1.23	1.29	**1.29**
Isolu	3.67	3.68	3.74	3.67	3.81	3.86	3.64	**3.73**
Leu	6.76	6.75	6.81	6.68	6.85	7.00	6.57	**6.78**
Tyr	3.22	3.19	3.23	3.21	3.28	3.33	3.18	**3.23**
PhenylA	4.38	4.39	4.45	4.34	4.57	4.73	4.26	**4.45**
Trypto	1.09	1.08	1.10	1.09	1.10	1.05	1.07	**1.08**
g protein/100g sample	86.18	85.75	86.93	85.77	87.24	88.67	85.23	**86.54**
Total g N /100 g sample	13.65	13.63	13.71	13.56	13.74	13.91	13.48	**13.67**
NCF	6.31	6.29	6.34	6.33	6.35	6.37	6.32	**6.33**

NCFs were calculated as described above for Soy Protein Isolates

Table 13: Calculation of Nitrogen Conversion Factors from Soy Flake anhydrous amino acid data

	g anhydrous AA residue/100 g sample					
AA	**2005**	**2005**	**2004**	**2005**	**2005**	**Averages**
lys	5.62	5.48	5.27	5.63	5.58	**5.52**
Hist	2.15	2.17	2.09	2.17	2.16	**2.15**
Arg	6.82	6.82	6.38	6.68	6.81	**6.70**
Asp	9.99	10.38	9.74	10.09	10.13	**10.06**
Thr	3.29	3.20	3.19	3.32	3.24	**3.25**
Ser	4.15	4.17	4.18	4.21	4.23	**4.19**
GlutA	17.55	17.72	17.75	17.79	18.08	**17.78**
Pro	4.47	4.26	4.23	4.41	4.45	**4.36**
Glyc	3.17	3.15	3.10	3.22	3.19	**3.17**
Ala	3.50	3.37	3.41	3.53	3.50	**3.46**
Cyst	1.30	1.34	1.23	1.26	1.21	**1.27**
Val	4.09	4.04	3.96	4.11	4.10	**4.06**
Meth	1.30	1.25	1.29	1.23	1.18	**1.25**
Isolu	3.67	3.68	3.64	3.66	3.70	**3.67**
Leu	6.58	6.58	6.56	6.62	6.69	**6.60**
Tyr	3.29	3.15	2.99	3.22	3.31	**3.19**
PhenylA	4.41	4.46	4.34	4.44	4.50	**4.43**
Trypto	1.11	1.07	1.13	1.19	1.10	**1.12**
g protein/100g sample	86.46	86.28	84.49	86.76	87.15	**86.23**
Total g N /100 g sample	13.72	13.67	13.32	13.74	13.8	**13.65**
NCF	6.30	6.31	6.34	6.31	6.32	**6.32**

NCFs were calculated as described above for Soy Protein Isolates