AOAC INTERNATIONAL STAKEHOLDER PANEL ON DIETARY SUPPLEMENTS

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Protein Working Group

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Sheraton Dallas Hotel, 400 N Olive Street, Dallas, Texas

Fitness for Purpose from 3/17

"Method must identify and quantify specific proteins in presence of other proteins and potential adulterants. Quantitative method must provide accurate and precise concentrations of specific intact proteins in ingredients and finished goods."

Protein Working Group Members

- Spencer Carter, Genysis Labs
- Joseph Betz, NIH
- Steven Dentali, Herbalife
- Jason Hendrickson, Bodybuilding.com
- Martha Jennens, Covance
- Suvash Kafley, Milk Specialties
- Adam Kuszak, NIH
- John Lawry, Covance
- Katerina Maastovska, Covance
- Elizabeth Mudge, BCIT
- Melissa Phillips, NIST
- Curtis Phinney, Consultant
- Catherine Rimmer, NIST
- Brian Schaneberg, Starbucks
- Aniko Solyom, GAAS Analytical
- Darryl Sullivan, Covance
- James Sullivan, Natures Products
- John Spzylka, Mérieux NutriSciences
- Barry Tulk, DuPont
- Robert Wildman, Dymatize
- Jason Wubben, ADM
- Jinchuan Yang, Waters
- Kurt Young, GNC
- Joseph Zhou, Sunshineville
- Garrett Zielinski, Covance

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Protein Working Group
Work to Date

- 1 In Person Meeting
- 3 teleconferences (March 2016 – June 2016)
- 4 SMPRs Drafted
- Public comment period (August, 2016)
- SMPRs made ready for SPDS review and approval

Background

- Proteins are polypetides made of individual amino acids in a linear chain
- Form the basis of life and perform functions in every system of the human body
    - Enzymes catalyze biochemical reactions
    - Hormones are used for cell signaling and communication
    - Synthesize and repair DNA
    - Tranport materials across the cell
    - Respond to stimuli
    - Provide structural support

Significance

- Estimated that 4 billion metric tons of food protein is produced globally
- Estimated that $94M was lost by changing the nitrogen-to-protein factor for dairy products from 6.38 to 6.25 in Europe in 2006
- Proteins make up $4.7B dollars in the Sports Nutrition industry, which represents 70% of the total revenue in that category

Adulteration

- Non-selective protein methods have fueled the potential to adulterate samples with non-proteins and give inaccurate results
- Melamine, urea, free amino acids cannot be differentiated using Kjeldahl, Dumas methods and have been the source of scandals
- Public health is still at risk; Economics still push adulteration

Existing Methods (Qualitative)

- Some proteins have FCC monographs. For example, Whey Protein is identified by testing for:
  - Ash
  - Fat
  - Lactose
  - Loss on drying
  - Nitrogen (and apply conversion factor)
- DNA Analysis
- LC/MS/MS

Existing Methods (Quantitative)

- Kjeldahl
  1. Wet digestion converts nitrogen to ammonium sulfate
  2. Neutralize to convert to free ammonia
  3. Distill ammonia into boric acid
  4. Back titrate with alkali
  5. Convert nitrogen concentration to protein using conversion ratio
  - "True Protein" can be determined by precipitating out protein, analyzing remaining nitrogen, subtracting from total nitrogen content

Existing Methods (con't)

- Dumas
  1. Combust samples at high temp with oxygen to form water, carbon dioxide, nitrogen
  2. Remove water and carbon dioxide using column
  3. Nitrogen is measured using a thermal conductivity detector
  4. Convert nitrogen concentration to protein using conversion ratio

Existing Methods (con't)

- Amino Acids
  1. Hydrolyze protein into amino acids
  2. Derivatize amino acids
  3. Determine protein by summing individual amino acids
- Dye-binding
  1. Form complex with dye and protein using ionic or electrostatic forces.
  2. Determine dye concentration using spectrophotometer

Existing Methods (con't)

- Copper-Binding
  1. Copper ions react with proteins to form complex
  2. Measure absorbance at 540 nm
- Others
  - UV absorption
  - Infrared

Challenges with Existing Methods

- Kjeldahl, Dumas: not selective to protein;
- True Protein Kjeldahl: non-protein, nitrogen-containing compounds may precipitate or form complex with precipitated protein
- Amino Acid: Inaccurate quantitation due to variable recovery of amino acids
- Copper, Dye-Binding: other constituents besides proteins form complexes
- Lack of Standards
  - Protein biosynthesis is expensive, time-consuming, not robust
  - Proteins samples vary widely and usually include multiple proteins

SMPR Key Points

- Definition of Protein is same as IUPAC: "Naturally occurring and synthetic polypeptides having molecular weights greater than about 10000 daltons (the limit is not precise)"
- Four SMPRs are being proposed:
  - Quantitative (i.e. Determination) and Qualitative (i.e. Identification)
  - Plant and Meat-Derived Proteins

Method Performance Requirements for Determination in Plant & Meat-Derived Proteins

Parameter	Criteria
Analytical Range (%)	0.1 – 100
LOQ (%)	0.050
LOD (%)	0.025
Recovery (%)	90-110 (0.1 – 1% range) 97-103 (>1 – 100% range)
% RSD_r	≤ 10 (0.1 – 1% range) ≤ 6 (>1 – 100% range)
% RSD_R	≤ 12 (0.1 – 1% range) ≤ 8 (>1 – 100% range)

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Single-Lab Method Performance Requirements for Identification in Plant & Meat-Derived Proteins

Study	Parameter	Parameter Requirements	Target Test Conc	Minimum Acceptable Results
Matrix	POI @ low conc	≥ 33 reps representing all listed target analytes	0.1 %	90% POI (95% confidence interval) of the pooled data for all target compounds and matrices.
	POI @ high conc	≥ 5 reps per matrix type spiked at 10x the designated low level target test conc	10%	100% correct analyses are expected
	POI @ zero conc	≥ 5 reps per matrix type	0 %	
Selectivity	False positive rate	Evaluate samples containing non-protein ingredients and listed adulterants	10 %	≤ 5%

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Multiple-Lab Method Performance Requirements for Identification in Plant & Meat-Derived Proteins

Study	Parameter	Parameter Requirements	Target Test Conc	Minimum Acceptable Results
Matrix	LPOI	Use ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods	0.1 %	≥ 0.85
			10 %	≥ 0.95
	LPOI (0)		0 %	≤ 0.05

SMPR Sources of Proteins

Plant-Derived

- Algae
- Canola (Rapeseed)
- Flax
- Hemp
- Pea
- Potato
- Pumpkin
- Quinoa
- Rice
- Soy
- Wheat

Meat-Derived

- Casein
- Egg
- Whey
- Milk

SMPR Non-Protein Ingredients Including Adulterants

- Melamine
- Urea
- Free amino acids
- Creatine
- Caffeine
- Taurine
- Surfactants
- Peptides (less than 10,000 daltons)

Comments Submitted

- Comment: “Though the given protein definition is recommended by IUPAC, it will have little practical use for the food industry. For instance, in the newly revised FDA food and supplement nutrition fact labeling, FDA recognizes all peptides as proteins (a merely amino acid source). And 10000 sounds over emphasized.”
- Proposed Change: “Polymeric chains of amino acid residues connected with peptide bonds.”

Motion

Move to accept the Standard Method Performance Requirements for:

- Determination of Meat Derived Proteins
- Determination of Plant Derived Proteins
- Identification of Meat Derived Proteins
- Identification of Plant Derived Proteins

….as presented.

Discussion?