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# USP Roundtable for DS Protein Standards

Hosted on February 07, 2017  
USP–U.S., Rockville, MD

# Discussion Agenda

- ▶ Identification Tests for Proteins from Various Sources
- ▶ Quantitative Determination of Proteins
- ▶ Determination of the Purity of Proteins
- ▶ Limits for Contaminants in Proteins
- ▶ Labelling, Packaging, Storage, and Handling

# Identification Tests for Proteins from Various Sources

## Current identification tests for proteins used in industry

- ▶ Comprehensive supplier chain qualification program helps reduce routine ID tests at the manufacturing site. Some manufacturers audit suppliers on a quarterly or annual basis.
- ▶ Typical identification tests: appearance, organoleptic, Kjeldahl, Near Infrared (NIR) for process monitoring and QC release.
- ▶ Amino acid profiling is used on a demand basis by customers.

## Suggested identification tests for proteins from various sources

- ▶ Manufacturers were aware of advanced tests: electrophoresis, CE, peptide mapping, mass spectrometry, ELISA for plant based proteins.
- ▶ Suggested that amino acid profiling in combination with protein profiling with electrophoresis (SDS PAGE) is feasible and suitable.

# Quantitative Determination of Proteins from Various Sources

## Current quantification tests for different sources

- ▶ The standard method for protein quantification in industry is Kjeldahl or combustion (Dumas).
- ▶ NIR is commonly used for protein quantification. Total amino acid (AA) contents is believed to provide accurate protein contents.

## Suggested quantification tests for protein ingredients and finished products containing proteins from various sources

- ▶ Suggested that Kjeldahl or Dumas is a widely accepted quantification method.
- ▶ Total Amino Acids (AA) can be used as a complementary method to Kjeldahl or Dumas. Total AA methods require further standardization and validation.

# Determination of the Purity of Proteins from Various Sources

## Impurities/specific tests for proteins

- ▶ Dairy protein industry routinely test for loss on drying (LOD), ash, fat and lactose.
- ▶ Some manufacturers test for non-protein nitrogen contents through precipitation or molecular weight filtration.
- ▶ The soy industry tests for fat and minerals.
- ▶ Rice proteins are tested for heavy metals (lead, and arsenic). Heavy metals in rice is a global concern.

## Suggested purity tests for protein ingredients from various sources

- ▶ Non-protein nitrogen contents, LOD, fat, ashes, lactose for dairy proteins

# Limits for Contaminants in Proteins from Various Sources

## Suggested tests for chemical contaminants in protein ingredients from various sources

- ▶ Mycotoxins (e.g. Aflatoxins), heavy metals, and pesticides for vegetable proteins
- ▶ Nitrogen containing compounds (e.g. Nitrile, Nitrate, Melamine, Cyanuric acid, Urea, Amidinourea, Ammelide, Ammeline, Biuret, Cyromazin, Dicyandiamide)

## Suggested tests for microbiological contaminants in protein ingredients from various sources

- ▶ Stakeholders select tests for microbiological contaminants based on HACCP and their own risk assessments depending on sources.
- ▶ Suggested total plate counts (TPC), yeast-molds, *E. coli* and *Salmonella*. In addition, *Listeria* for dairy protein manufacturers.

## ▶ Identification

- Should be specific to various sources and processes
- Orthogonal approach (candidates)
  - Protein profiling (SDS PAGE)
  - Amino acid profiling

## ▶ Assay

- Should address the true content of proteins.
- Orthogonal approach (candidates)
  - Non-specific nitrogen determination (Kjeldahl or Dumas)
  - Total amino acid contents

## ▶ Limits for Contaminants

- Should consider risk mitigation approaches & specific acceptance criteria.
  - Heavy metals
  - Potential adulterants (Nitrogen containing compounds)



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