

Food Protein Workshop: Developing a Toolbox of Analytical Solutions to Address Adulteration

USP Headquarters, Rockville, Maryland
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Summary, Recommendations and Actions Breakout B



Overview: Key initial points

- Interest in retaining nitrogen method for trade purposes
- Categorize techniques
 - Purity assessment
 - Rapid screening/authentication
 - Confirmatory
- Need for reference samples/standards; authentic samples for database
- Recognize that melamine is just one low molecular weight, high nitrogenous compound



Eric Eccleston: A Review and Critique of the Current Technologies for Protein Quantitation

- Using amino acid analysis as a triage approach to protein estimation and abnormal AA ID
 - Fastest version is 20? minutes hydrolysis (liquid phase), then chromatographic separation, about one hour total elapsed time
 - Could compare results to nitrogen-based method
 - Could look for something abnormal
 - Screening method



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Jürgen Möller: Status of Nitrogen-based Methods (Kjeldahl/Dumas)

- Kjeldahl measures organic nitrogen and ammonia
- Dumas measures total nitrogen, including inorganic fraction (including NO_2/NO_3)
 - Therefore, Dumas gives higher value for some products, e.g., lettuce.
- Multiple fractionation schemes as sample preparation (pepsin digests, non-protein nitrogen)
- Nitrogen assays closely linked to international commerce



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D.M Barbano: A Kjeldahl N-based True Protein Method that Accounts for 12% TCA Soluble Non-protein Nitrogen

- Use TCA precipitation and filtration to get precipitate as true protein nitrogen (i.e., to get protein away from other nitrogenous compounds; a pre-treatment)
- Used in dairy industry since true protein is basis for payment, feeding managing, and record keeping for genetic stock
- Has been used in other industries (e.g., soy)
- TCA precipitation approach also works for dry milk
- Melamine up to approximately 0.6 g/L shows up as NPN (not true protein). Above that, additional rinsing is required.



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Sam Chang: Status and Future of Colorimetric Methods for Protein Measurement

- Biuret, Lowry, Dye Binding, BCA
- Should not measure melamine as protein (but generally not confirmed)
- Require comparison to nitrogen-based methods or reference protein standards
- All have some interferences
- Automated: CEM Sprint anionic dye binding
 - Above some level, melamine is detected as protein



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Peter Scholl: Would Application of Proteomic Technologies to Food Analysis Create Useful Data?

- Proteomics has potential as discovery tool in food analysis to figure out which proteins don't belong (compared to reference sample)
 - Can detect unknown or subtle differences
- Applications: Public health incidents, surveillance
- Techniques: DIGE, iTRAQ, label free MS methods
- Don't know effect of food matrix
- Disadvantages: Time consuming; technically challenging; expensive; low throughput



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Kevin Shefcheck: Proteomics in Food Protein Analysis

- Allergen detection: ELISA, Bioplex, SPR (all are antibody-based screening)
 - Target is final finished product samples
 - Problems: cross-reactivity and false positives
 - Confirm with LC/MS
- LC/MS challenges:
 - **Sample preparation:** extraction and cleanup
 - Using immunoaffinity columns for cleanup
 - Use internal standards to manage extraction, digestion, and quantitation



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Harvey Indyk: Food Protein- What role for SPR biosensors

- Bottom line for companies:
 - Food safety <-> Brand protection <-> Profitability
- Biosensor for protein and adulteration detection
 - Have potential for selectivity/specificity and sensitivity
 - Not measuring total protein, only “reactive” protein (immunologically active, e.g., undenatured, native, intact IgG)
 - Major issue is non-specific binding – need to characterize
 - Applications: milk adulteration with plant protein, species adulteration for milk, bovine IgG, melamine



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Eric Eccleston: What Proteomics Has to Say About Protein Quantitation, Nutrition and Adulteration

- Proteomics provides:
 - Kits, etc. for sample preparation and protein separation
 - Pre-packaged, cheap, robust methodology
 - Examples: IEF strips and precast SDS gels to do 1-D or 2-D gel electrophoresis
 - Can use literature as starting point for databases



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Basic methods in the “toolbox”

- Fractionation
- Kjeldahl
- Dumas
- Colorimetric?



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Advanced methods in the “toolbox”

- AA analysis
- N-terminal analysis
- Microfluidics
- Capillary electrophoresis
- Reverse phase HPLC
- Ion exchange chromatography
- Electrophoresis
- Immunoassay (ELISA, SPR...)
- Mass spec



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Infrared

- Mid-IR (fixed wavelength)
 - Most used method in dairy industry for fluid milk
 - Measures melamine as protein
- PLS-full spectrum
 - Will have varying sensitivities to adulteration



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Breakout B—Overarching

- Fractionation approach could be pursued with matrices other than just milk
- Need quick screening test to authenticate (i.e., see if something is abnormal)
 - How to determine if abnormal?
 - For chemometric-driven methods, data for “normal” samples needs to be constantly fed into calibration data set; need to prevent “toxication” of calibration samples
- Focus testing on ingredients (vs. finished product)
- Supply chain management and traceability (GAP-Good Agricultural Practices)
- Education and vigilance



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