Developing a Targeted Parallel Reaction Monitoring Method for the Detection of Milk-Derived Ingredients in Complex Matrices

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OVERVIEW

- Milk proteins are important food allergens to detect and quantify, but they are often used in food products as various milk-derived ingredients that may predominantly contain certain protein fractions, making development of a universal detection method challenging.
- **Objective**: Utilize discovery proteomics analysis of milk-derived ingredients to select target peptides for a broadly-applicable milk protein detection method

INTRODUCTION

- Milk allergy is one of the most common food allergies, affecting about 1.7% of children.[1]
- U.S. and European regulations require that milk and any ingredients derived from milk must be declared on food labels. Numerous different milk-derived ingredients are used by the food industry and represent a variety of milk protein fractions.
- Undeclared milk presents a substantial health hazard, and quantitative detection methods are critical to detect undeclared milk proteins.

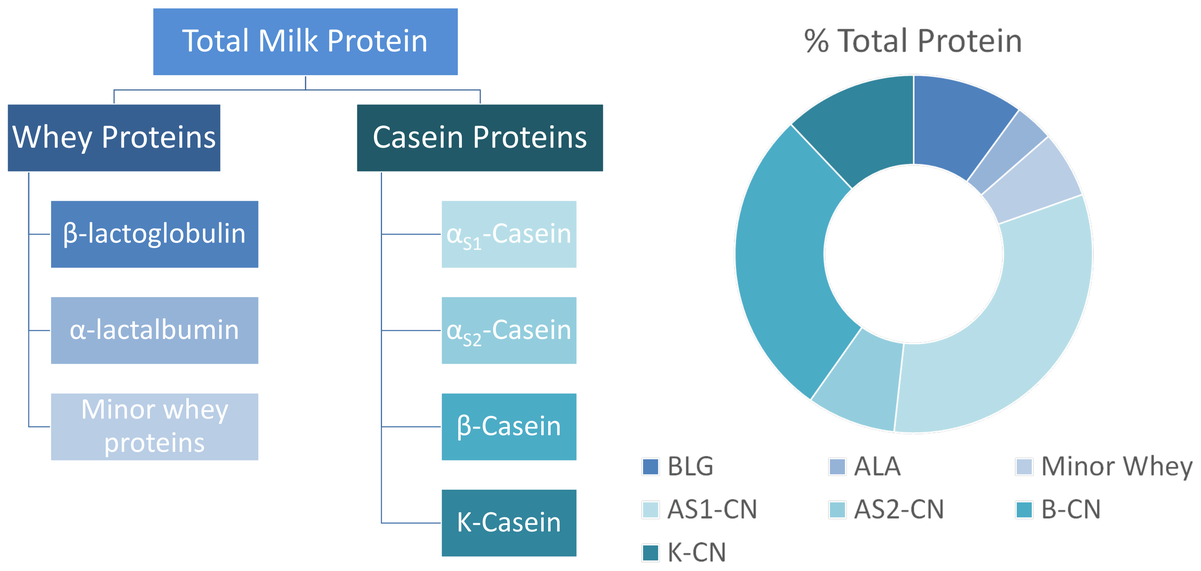

Figure 1: Milk Protein Fractions and Total Protein Composition

MATERIALS AND METHODS

Milk-Derived Ingredients

Table 1: Total Protein Content of Milk-Derived Ingredients (Dumas Method)

Ingredient	Protein Content (%)
Nonfat dry milk	34.97
Acid whey	10.30
Sweet whey	11.26
Whey protein concentrate 34	31.33
Whey protein concentrate 80	79.45
Sodium caseinate	89.72

Extraction

Milk ingredient in buffer (1:9 w:v) (50 mM Tris-HCl, pH 8.6, with 6 M Urea, 20 mM DTT, and 1% PVPP) → 60 °C, 10 min → Sonicate: RT, 10 min → 60 °C, 10 min → Centrifuge: 13,000 x g, 10 min → Obtain supernatant → In solution digestion

Tryptic Digestion and LC-MS/MS

Sample Extract → Reduction → Alkylation → Digestion → C18 Clean-up → LC-MS/MS (Thermo Scientific™ Q Exactive™ MS): DDA Discovery or PRM Mode

RESULTS

Discovery Proteomics

Table 2: Number of High-Confidence Peptide Identifications for Major Milk Proteins Observed in Different Milk-Derived Ingredients.

	NFDM	Acid Whey	Sweet Whey	WPC 34	WPC 80	Sodium Caseinate
Alpha S1-Casein	25	13	12	15	31	28
Alpha S2-Casein	28	10	8	16	32	35
Beta-Casein	11	7	7	7	13	14
Kappa-Casein	8	5	1	1	10	10
Beta-lactoglobulin	21	20	23	29	28	17
Alpha-lactalbumin	16	14	15	17	15	14

- Discovery data analyzed using Thermo Scientific™ Proteome Discoverer™ 2.1 software (SEQUEST® HT search)
- Database: all *Bos taurus* entries in UniProt®, plus common contaminants
- High confidence determined with Percolator, FDR < 0.1%.
- While casein peptides were found much more frequently in NFDM, WPC 80, and sodium caseinate, whey protein peptides (from BLG and ALA) were observed across the different ingredients.

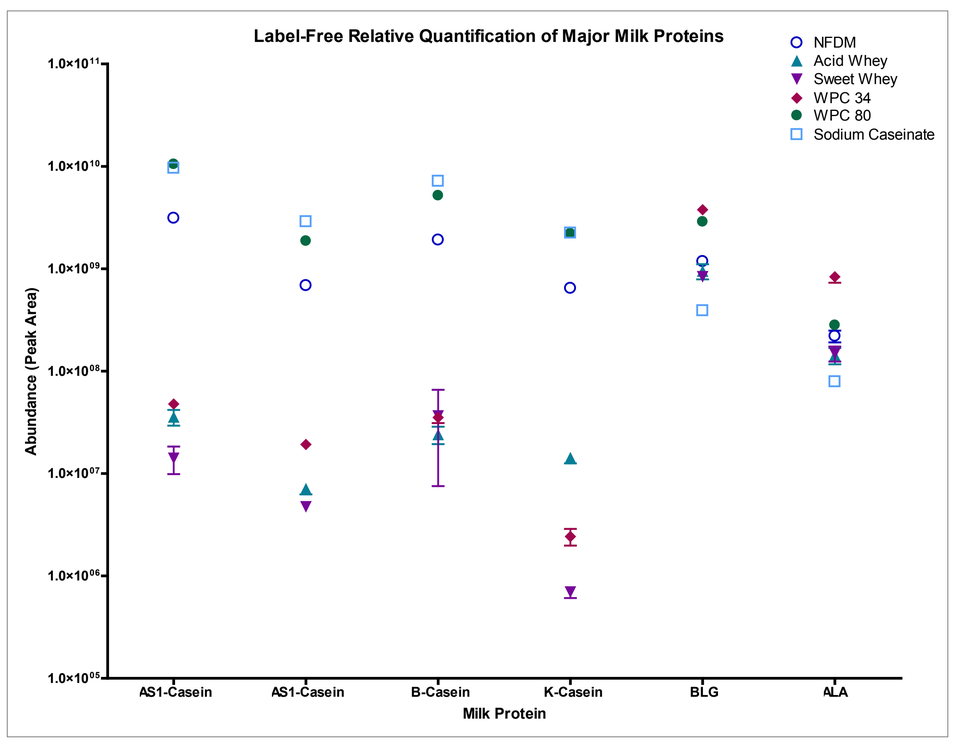

Figure 2: Summary of Label-Free Relative Quantification at the Protein Level. Symbols indicate mean protein-level peak area and error bars indicate SEM.

- Label-free quantification performed with Proteome Discoverer 2.1 software (precursor ion area measurement)
- Similar to the peptide identifications, the casein proteins delivered a quite broad distribution of relative quantitative values among the different ingredients.
- The whey proteins (BLG and ALA), however, had much more similar quantification data across the range of ingredients analyzed.

FUTURE WORK

- Prepare incurred foods (cookies) with various milk ingredients and analyze with targeted PRM method.
- Develop quantification strategy for milk protein from different milk-derived ingredients.

Preliminary Targeted Proteomics: Parallel Reaction Monitoring (PRM)

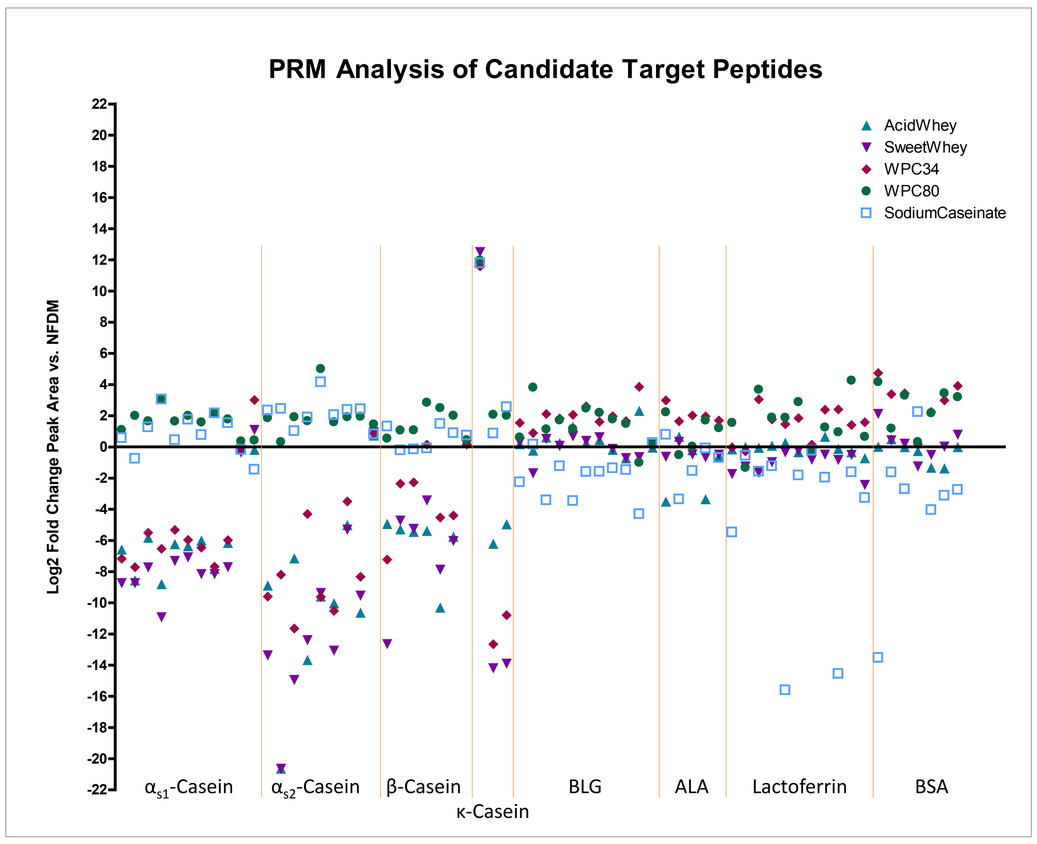

Figure 3: Targeted Parallel Reaction Monitoring of Candidate Target Peptides. Symbols indicate the Log2 of the fold-change of individual peptide peak area in the ingredient vs. the NFDM sample.

- Preliminary PRM analysis of candidate target peptides showed similar trends as the untargeted analysis:
  - Peptides from casein proteins were generally more abundant in sodium caseinate and WPC 80 than acid whey, sweet whey, or WPC 34.
  - Whey peptides (from BLG, ALA, lactoferrin, and BSA) were more uniformly abundant across the different ingredients.
- Candidate target peptides were further refined based on their performance, for use in a method to detect milk proteins in food matrices.

CONCLUSIONS

- The identification and quantification of peptides from various milk proteins was dependent on the milk-derived ingredient source.
- Casein proteins (and peptides) were more abundant and delivered more identifications in NFDM, sodium caseinate, and WPC 80. Whey proteins (and peptides) were observed at more similar abundances in all ingredients than the casein proteins and peptides.
- Developing a quantitative method to deliver a single result for total milk protein concentration from any (unknown) ingredient source will be difficult due to the differences in individual protein and peptide concentrations in various milk-derived ingredients. Units of total milk protein are, however, the most relevant for quantitative risk assessment and comparisons with clinical threshold data.

ACKNOWLEDGEMENTS

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Mass spectrometry was conducted on a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer placed at UNL.

Reference

1. Gupta, R.S., et al., Pediatrics, 2011. **128**: p. e9-e16.