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INTRODUCTION

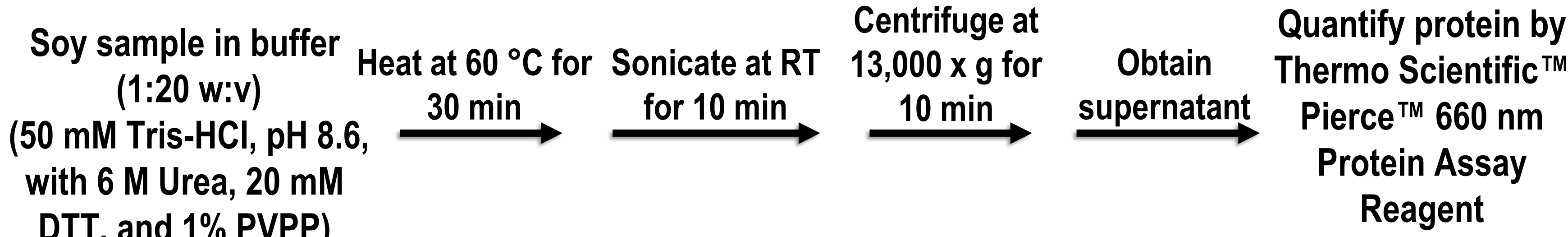
- Soybeans are one of the “Big eight” allergenic foods in the US, with soy allergies affecting approximately 0.4% of children.¹
- Because of their functional properties and high protein content (~38%), soybeans have been widely used in various food products such as baked foods (e.g. cookies, crackers), beverages, and meat products in the forms of soy flour, soy protein isolates, and soy protein concentrates.
- Mass spectrometry (MS) is playing an increasingly important role in allergen detection and quantification since it has shown better performance in allergen quantification, in some cases, compared with other technologies such as ELISA.
- The objective of the current study was to identify and evaluate target peptides from a variety of soy-derived ingredients for the parallel reaction monitoring (PRM) detection of soy protein in foods.

MATERIALS AND METHODS

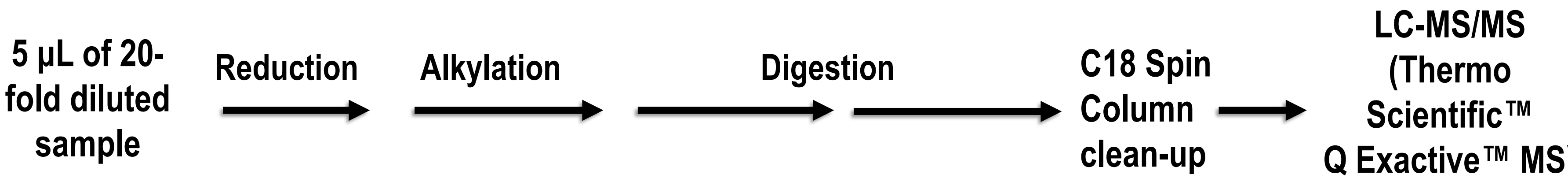
Soy ingredients information

| Acronym | Product type | Processing method | Composition |
|---------|-------------------------|--------------------------|-------------|
| NRSF | Non-roasted soy flour | Minimally heat processed | |
| RSF | Roasted soy flour | Fully heat treated | |
| SPI-A | Soy protein isolate | Hydrolyzed | |
| SPI-B | Soy protein isolate | | |
| SPC-A | Soy protein concentrate | | |
| SPC-B | Soy protein concentrate | | |

Extraction method



Tryptic digestion and LC-MS/MS



RESULTS

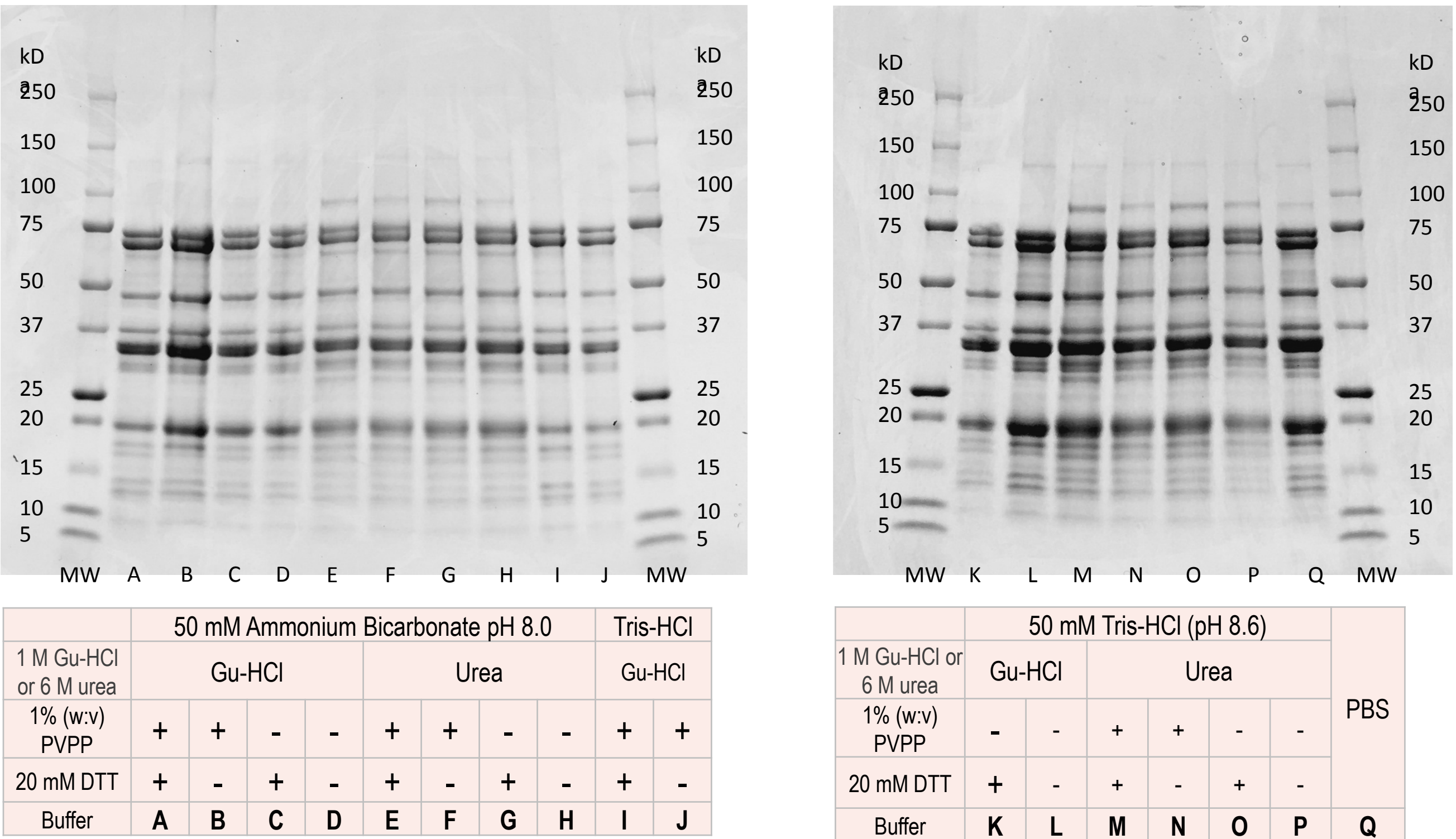


Figure 1. SDS-PAGE comparison of protein extraction efficacy from unroasted soy flour

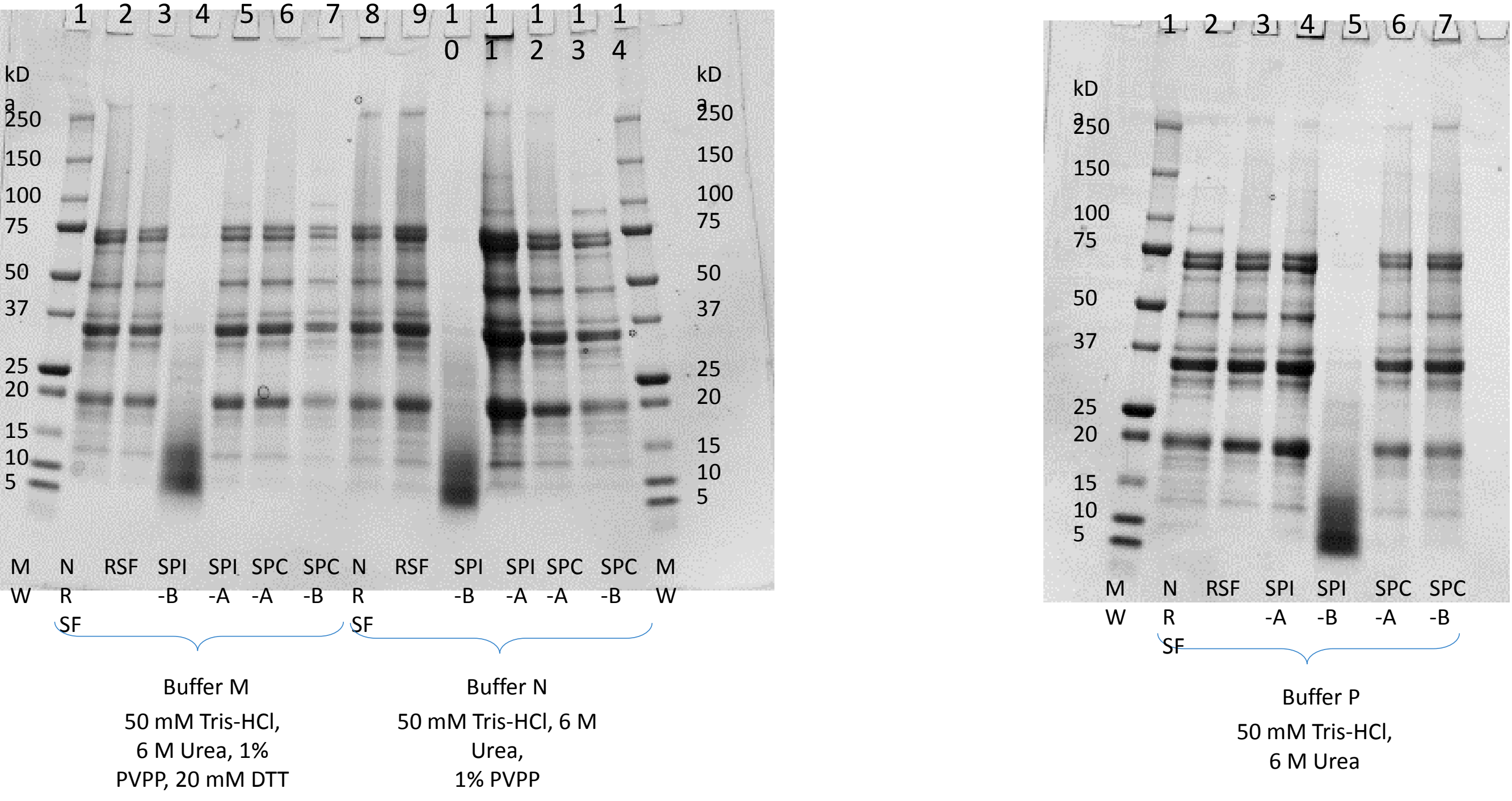


Figure 2. SDS-PAGE gel of six commercial soy ingredients with three extraction buffers

Criteria for target peptides selection:

- belongs to major seed storage proteins
- does not contain methionine
- 6-25 aa
- high confidence
- the standard deviation of peak area between all soy ingredients is less than 1.5
- unique amino acid sequence
- conserved among protein isoforms
- have good performance at low concentration

a

| Protein | Sequence | Contain Cysteine? | Contain Methionine? | Soy-specific peptide? | number of soy ingredients can detect the peptide | Identified as a potential target peptide? |
|---------------------------|-----------------------|-------------------|---------------------|-----------------------|--|---|
| 2S albumin | ELINLATMCR | Yes | Yes | Yes | 6 | |
| 2S albumin | KQLQGVNLTPEK | Yes | | Yes | 6 | Yes |
| Basic 7S globulin | LMVFDLAR | | Yes | Yes | 6 | |
| Glycinin G2 (A2B1a) | GKQEEENEGSNLGSFAPEFLK | | | Yes | 5 | |
| β-conglycinin alpha chain | LITLAIPVNKPGK | | | Yes | 6 | Yes |

b

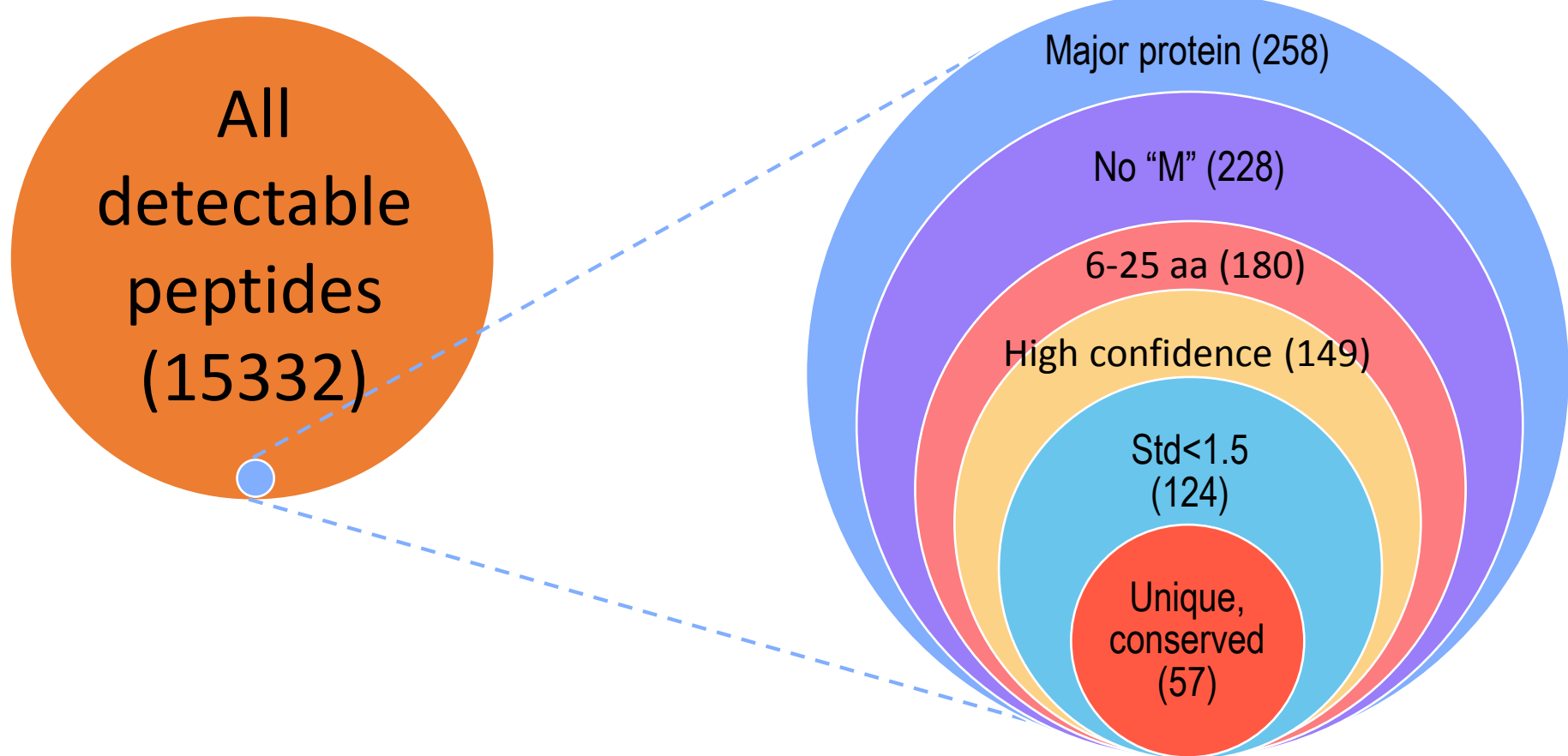


Figure 3. Target peptide selection a. Example of target peptide selection b. Visualization of target peptide identification for soy protein, number of peptides is given in brackets

OVERVIEW

- Comparison of protein profiles of six commercial soy ingredients
- Selection of target peptides for the soy ingredients
- Comparison of abundance of the selected peptides across six soy ingredients

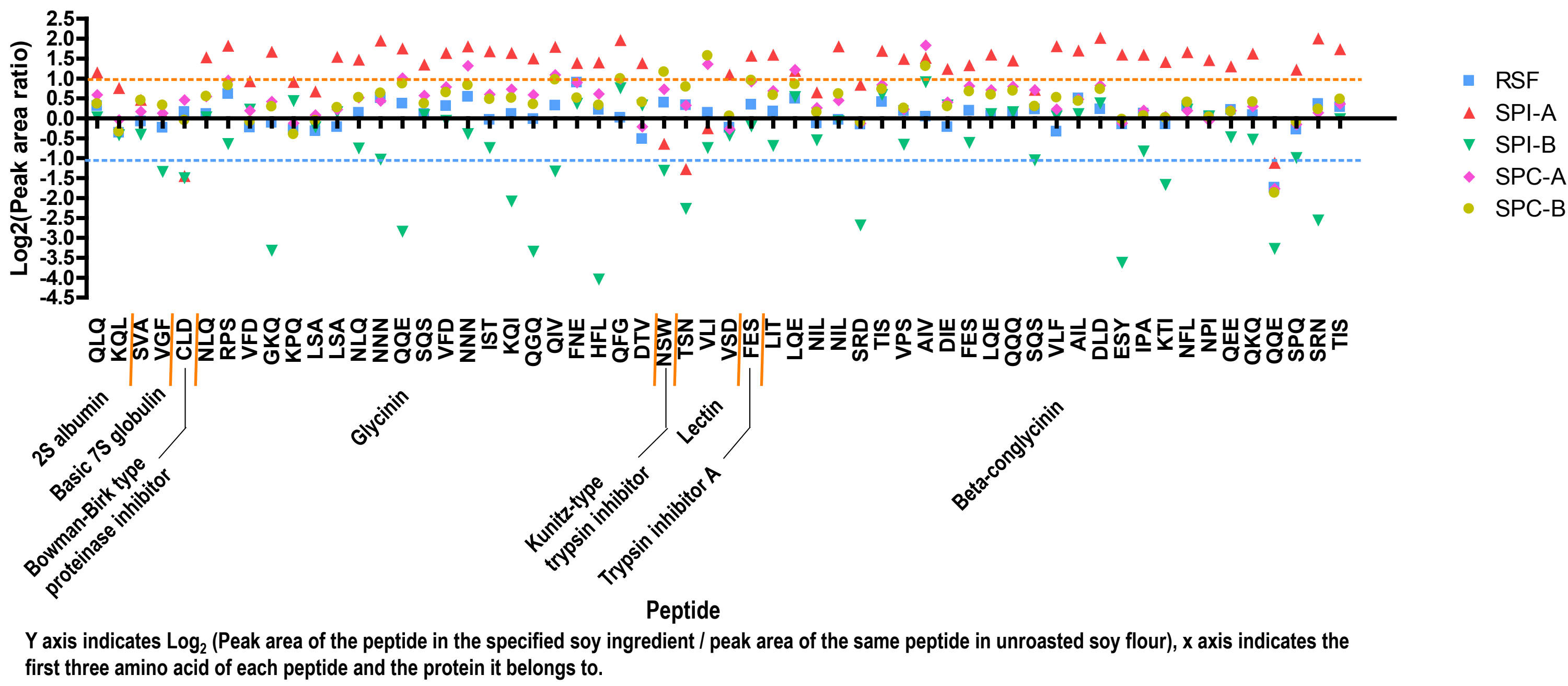


Figure 4. Comparison of abundance of selected peptides across six soy ingredients

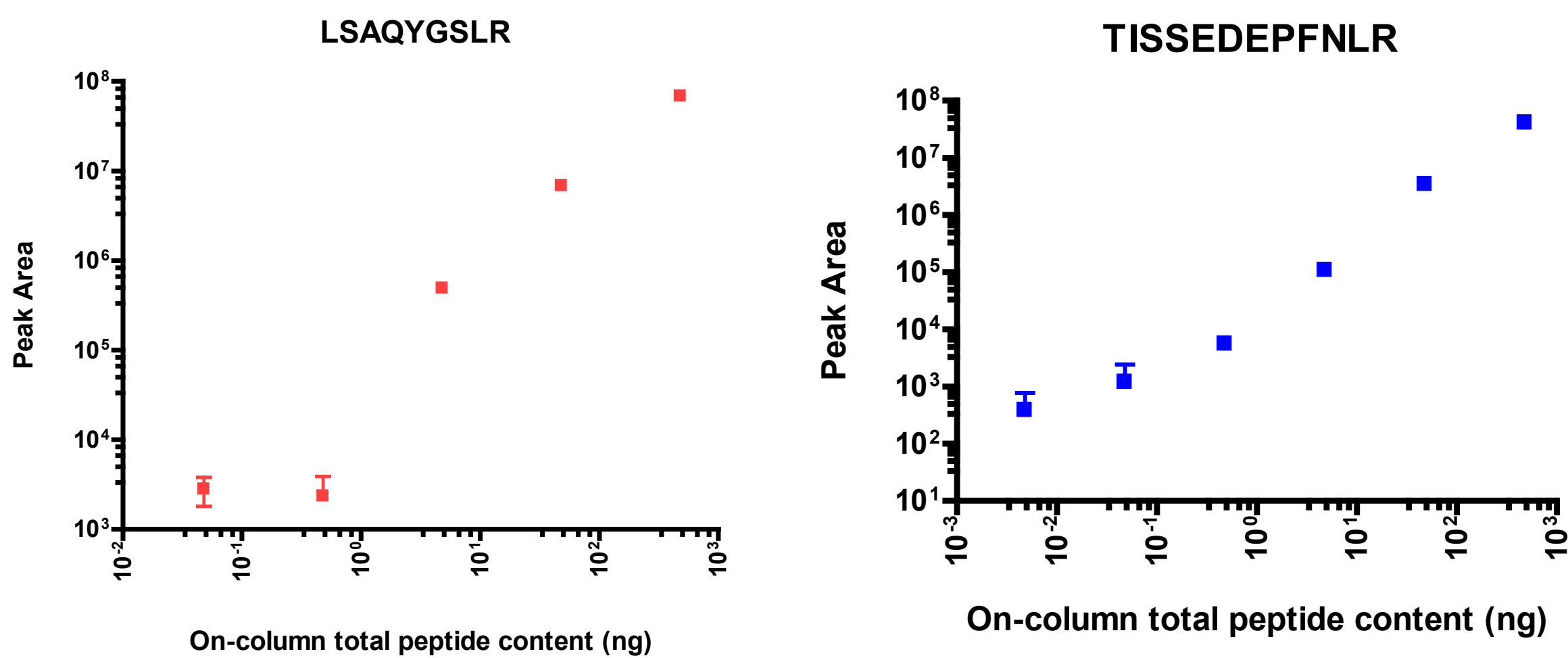


Figure 5. Peptide performance at each level of on-column total peptide content (sample: NRSF, two soy digests with duplicate injections)

CONCLUSIONS

- Buffers M, N, P (50 mM Tris-HCl with 6 M Urea) have shown the optimal extraction effect among all of the buffers.
- The protein profiles of most soy ingredients are very similar to each other except for the SPI-B (soy protein isolate-B), which contains mostly low molecular weight peptides.
- 57 peptides that satisfy a set of pre-determined criteria were selected for future targeted MS analysis.

FUTURE STUDIES

- Optimize extraction and digestion methods for the food matrices test.
- Evaluate quantitative performance of the selected target peptides.
- Develop a quantitative Parallel Reaction Monitoring (PRM) method using isotopically labeled internal standards.

Reference:

1. Savage, J. H., Kaeding, A. J., Matsui, E. C. and Wood, R. A. The natural history of soy allergy. *J. Allergy Clin. Immunol.* **2010**, 125, 683–686.

ACKNOWLEDGEMENTS

This research is part of a collaboration between FARRP/UNL and Thermo Fisher Scientific. Mass spectrometry was conducted on a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer placed at UNL.