

## **PW14 Peptide size distribution profiling of milk protein hydrolysates**

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Peptides, size distribution profiling, protein hydrolysates, MWP

An accurate method for size distribution profiling of peptides in hydrolysates was developed.

Size exclusion chromatography is widely used to obtain the size distribution of peptides in food protein hydrolysates. Such a profile is commonly called a molecular weight profile (MWP) within the dairy industry. The MWP provides an indication of the potential allergenicity risk and functionality of the hydrolysate. There is no standard method for determining the MWP of hydrolysates and it is known that different methods can give significantly different results for the same sample.

We required an accurate, reproducible method to characterise our milk protein hydrolysates, in particular those used in infant formulas. We investigated the effects of a range of common size exclusion method variables, including pH, salt type and concentration, presence/absence of chaotropic agents and organic solvents, on the size distribution profiles of the hydrolysate ingredients themselves and of the peptide material extracted from infant formulae. Mass spectrometry was used to determine the masses of peptides present in fractions collected from the HPLC runs corresponding to four mass ranges (<1 kDa, 1-5 kDa, 5-20 kDa and >20 kDa). By comparing the actual masses of peptides in each fraction with the mass range predicted from the size exclusion calibration curve (log of standard peptide/protein mass versus retention time) we were able to determine which size exclusion-HPLC conditions provided the most accurate and reproducible size distribution profiles. These profiles constitute new MWPs for our industry.