**Unveiling the effect of whey protein hydrolysates on gut microbiota**

G Di Filippo1, S Calligaris1, M Marino1, A Rossi1, N Renoldi1, F Marroni1 and N Innocente1

*1* *Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine, Italy*

The gut microbiota is essential for human health, influencing metabolic functions, immune responses, and overall homeostasis. While dietary carbohydrates have been extensively studied for their impact on gut microbiota, the role of dietary proteins, particularly whey proteins, is less explored. Recent research indicates that bioactive peptides, generated during gastrointestinal digestion, can beneficially modulate the gut microbiota and promote the production of metabolites such as short-chain fatty acids (SCFAs)1. To harness these benefits more effectively, controlled enzymatic hydrolysis of whey proteins before gastrointestinal digestion has been proposed. This approach allows for the production of specific peptides with desired sequences and sizes, enhancing their bioactivity and possible gut microbiota modulation. At the same time, it improves protein digestibility and reduces allergenicity, making whey protein hydrolysates suitable for hypoallergenic diets2.

This study explored the impact of whey protein isolate (WPI) enzymatic hydrolysis on gut microbiota composition and metabolite production through an *in vitro* colonic fermentation model of whey proteins. Whey protein hydrolysates (WPHs) were produced using Alcalase and Protamex enzymes at varying hydrolysis times (10, 30, and 120 minutes). The samples were subjected to *in vitro* digestion using the standardised INFOGEST static methodology and then the undigested fractions of whey protein isolate (WPI, control) and WPHs were fermented. During digestion, hydrolysis enhanced the release of essential amino acids, including proline and asparagine, with distinct patterns depending on the enzyme employed, while preserving branched-chain amino acids crucial for muscle protein synthesis and energy metabolism. In the gut, WPHs promoted the growth of beneficial bacteria, such as *Acidaminococcus intestini*, while reducing pro-inflammatory species like *Dorea longicatena*, particularly in hydrolysates produced with Protamex. Fermentation of WPHs led to increased SCFAs, notably acetate and propionate, which are vital for gut and systemic health. Metagenomic analysis highlighted notable shifts in microbiota composition, with enriched populations of *Bifidobacterium adolescentis* and *Bifidobacterium longum*, two probiotics known for SCFA production, anti-inflammatory effects, and gut barrier enhancement. These changes were particularly evident in hydrolysates obtained after 10 and 30 minutes of hydrolysis with Alcalase.

These findings emphasised the potential of WPHs as bioactive substrates with applications in gut health. By tailoring enzymatic hydrolysis protocols, it is possible to enhance both digestibility and microbiota modulation, paving the way for the development of functional food products that support overall health and well-being.

*References:*

1 Wu, S., Bekhit, A.E.D.A., Wu, Q., Chen, M., Liao, X., Wang, J. and Ding, Y. (2021). Bioactive peptides and gut microbiota: Candidates for a novel strategy for reduction and control of neurodegenerative diseases. *Trends in Food Science & Technology*, *108*, 164-176.

2 Feng, C., Tian, L., Hong, H., Wang, Q., Zhan, X., Luo, Y. and Tan, Y. (2022). *In vitro* gut fermentation of whey protein hydrolysate: an evaluation of its potential modulation on infant gut microbiome. *Nutrients*, *14*, 1374.