

## GASTROINTESTINAL DIGESTION ENHANCES THE VASORELAXANT ACTIVITY OF A WHEY HYDROLYSATE

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Whey proteins present encrypted fragments with biological activity called bioactive peptides, which may be released from the parent protein through enzymatic hydrolysis and/or during gastrointestinal digestion. Their antihypertensive potential is the most studied bioactivity, which may be explained by the high prevalence of this chronic degenerative disease. This study aimed to investigate the molecular alterations generated during the gastrointestinal digestion and its consequences on the vasorelaxant potential of a whey hydrolysate. A whey protein concentrate 88% solution (1.25% w/v) was partially hydrolyzed by pepsin during 3h (pH2, 37°C), then it was spray dried. The powder was suspended (8% w/v) and submitted to an *in vitro* gastrointestinal digestion. Protein digestion was evaluated through the release of NH<sub>2</sub> groups and SDS-PAGE. Digested peptides were identified by nano-RSLC-MS/MS. Cumulative concentrations of undigested (1, 3, 5, and 10 mg.mL<sup>-1</sup>) and digested (1, 3, 10, 30, 50 and 100 µg.mL<sup>-1</sup>) whey hydrolysates were applied into phenylephrine pre-contracted rat aortic rings to measure their vasorelaxant capacity. A small degree of hydrolysis was achieved during gastric digestion, and more intense proteolysis was observed in the intestinal phase. The NH<sub>2</sub> concentration ranged from 12.4 mg.g<sup>-1</sup> to 24.2 mg.g<sup>-1</sup>. Complete β-lactoglobulin hydrolysis was observed on the electrophoresis gels after 10min of intestinal digestion. 252 peptides were generated during digestion, among which 3 peptides present angiotensin-I converting enzyme inhibitory activity and 33 are encrypted within other identified sequences. Biological assays showed that digestion potentialized the vasodilatory effect of the peptides, achieving 71.3%, while undigested whey concentration needed to be increased in 50 times-fold to achieve similar results. The *ex-vivo* assays also showed that undigested peptides also act via mechanisms independent of endothelium, while the digested ones are endothelium-dependent. Molecular changes occurred during gastrointestinal digestion altered the mechanisms of action and increased the vasorelaxant potency of the whey peptides.

Keywords: whey peptides; digestion; hypertension.