

HPLC FRACTIONATION OF PEPTIDES OBTAINED AFTER PARTIAL HYDROLYSIS OF A WHEY PROTEIN ISOLATE

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Whey protein isolates contain over 90% protein that can be converted into peptides of functional interest depending on the hydrolysis procedures, and enzymes specificity. The aim of this study was to fractionate through HPLC whey peptides obtained after partial hydrolysis using commercial, non-immobilized trypsin and pepsin. The experiments were done according to the enzymes manufacturer recommendations, and carried out for 5 hours. Chromatography was performed on a Shimadzu system (LC-20AD), with a UV detector and a C18 column. The mobile phase was a gradient (5 to 90%) with solvent A (0.1% TFA in H₂O), and B (0.1% TFA in ACN), in a flow rate of 0.4mL/min, at a running time of 30min. Detection was done at 216nm. Comparing the chromatograms of the tryptic (TRYP-HY) and peptic (PEP-HY) hydrolysis along the time, it was possible to observe that in both cases, the main reactions occurred after the first hour, with the appearance of more than 50 peaks in each chromatogram. When comparing the treatments, it was possible to observe that PEP-HY was able to generate more peaks, while TRYP-HY, showed less peaks, but more intense ones. For the TRYP-HY a higher degree of hydrolysis of the main whey proteins after 1-h experiment was observed, while PEP-HY did not completely hydrolyzed them, even after 4-h experiment. In conclusion, the fractionation of the peptides was efficient in the evaluated method, and the enzyme choice in a process will depend on the application, once they seem to afford different peptide profiles. Supported by FAPERJ.