Whey proteins are well known for their nutritional value, and their peptides produced by partial hydrolysis are noted as functional molecules, with recognized biological properties and different applications. In this way, optimizing the hydrolysis conditions is crucial for obtaining such molecules. The aim of this study was to investigate the enzymatic hydrolysis profiles of a whey protein isolate using commercial, non-immobilized trypsin and pepsin. The experiment was performed within 5 hours, with controlled pH and temperature. The degree of hydrolysis was measured by evaluation of tyrosine content and protein quantitation. Protein profile was monitored by electrophoresis (SDS-PAGE). The hydrolysis conditions were set according to the enzymes manufacturer. The trypsic fraction showed an increase of tyrosine content of 1.6-fold after 1h, while an increase of 6-fold was observed after 3-h hydrolysis. The protein content decreased showing a reduction of 50%, 54%, and 63% after 1, 3, and 4-h experiment. The data from 2, and 5-h experiments were not significantly different. The peptic fraction showed best results between 1 and 3-h hydrolysis, as an increase in tyrosine content was of 2.2-fold after 1-h hydrolysis, and of 3.5-fold after 3-h experiment. After 2-h hydrolysis, the protein content decreased 75%. The electrophoresis gel (SDS-PAGE), showed that both enzymes were active in the tested conditions in a large number of proteins from whey. In conclusion, these results are interesting since the hydrolysis rates of whey proteins were high after only 2-h experiments, with the added advantage of no further sample pre-treatment. Supported by FAPERJ.