UNIVERSITÀ DEGLI STUDI DI SALERNO

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Relazione Triennale

Isolation, characterization and biological evaluation of bioactive compounds from complex matrices with high nutraceutical value

Dottorando

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My PhD project based its main objective on the isolation, the characterization and the biological evaluation of bioactive compounds deriving from food matrices of animal and vegetable origin typical of the Mediterranean Area. During the first year, the research activity was focused on the identification of bioaccessible peptides generated as a result of a simulated gastrointestinal digestion process of six commercial buffalo milk dairy products. The scientific approach allowed us to identify 342 peptides deriving from the hydrolysis of caseins and whey protein of buffalo milk. However, as far as we know, only one third of the identified peptides reported a biological activity in the literature, so we conducted a rational biological characterization of the six gastro-enteric digests under study. In this regard, the six digested gastro-enteric buffalo-based milk were tested on intestinal epithelial cells (IEC-6) under induced oxidative stress conditions by using hydrogen peroxide. Among all the matrices investigated, at the maximum concentration tested, the buffalo Ricotta determined an increased reduction in the release of Radical oxygen species (ROS). In order to identify the main peptide molecules responsible for the biological activity, the GI digest of buffalo Ricotta has been simplified using both membrane filters with cut-off lower than 1 kDa and the use of semi-prep RP-HPLC. The obtained fractions were tested in the experimental conditions described above and the most active fractions were subjected to high-performance liquid chromatography experiments coupled with high-resolution mass spectrometry studies, through which it was possible to identify two peptides: BRP1 and BRP2 deriving from hydrolysis of β-lactoglobulin. Both peptides were able to reduce the release of ROS by determining the nuclear translocation of NRF-2 and increasing the expression of cytoprotective enzymes regulated by itself. Based on the results obtained during my second year of the PhD, the research activity was focused on the study of the bioavailability of the BRP1 and BRP2 peptides, using, for this purpose, Caco-2 cell monolayers. The results obtained showed that unlike BRP1, the BRP2 peptide permeated through the cell monolayer mainly using a passive diffusion mechanism. From *ex vivo* biological assays, the peptide showed an antioxidant and hypotensive activity through the reduction of the Ang II-induced vasoconstriction process, and by both the decrease of NADPH-oxidase activity and with the nuclear translocation of Nrf-2, that on the other hand, increased the expression of cytoprotective enzymes. The research activity of my third year of the PhD was focused on the synthesis and subsequent biological evaluation in *ex vivo* and *in vivo* experimental models of the most abundant peptide identified of the gastro-enteric digested buffalo milk ice cream. This matrix from previous *ex vivo* studies has determined a reduction in ROS release. Thus, in order to identify the main peptide molecules responsible of the biological activity observed, my research activity focused on the most abundant pentapeptide (PG1) identified in GI of buffalo ice cream. In order to ensure a sufficient amount of peptide for further biological tests in *ex vivo* and *in vivo,* the PG1 peptide was synthesized by solid phase peptide synthesis experiments in according to the Fmoc strategy. The next phase of the study was centered on the evaluation of the pharmacokinetic properties of the PG1 peptide. In particular, the pentapeptide showed good stability to digestive and microsomal enzymes. The bioavailability of the PG1 peptide was again evaluated using a Caco-2 cell monolayer and the results obtained showed that the peptide permeated the cell monolayer, mainly using a passive diffusion mechanism. We moved to evaluate the local activity of the PG1 peptide, through *ex vivo* studies conducted on mesenteric arteries, to evaluate a potential reduction in the Ang II-induced vasoconstriction process. The results showed that at increasing doses of Ang II the vasoconstrictor response was inhibited in a dose-dependent manner after pretreatment with the PG1 peptide. The next phase of the study then included an *in vivo* evaluation for peptide. Specifically, the efficacy of the peptide on wild type mice under conditions of Ang II-induced hypertension was evaluated. The results obtained showed that the administration of the PG1 8mg / Kg peptide for 14 days, via gavage, determined a normalization of blood pressure values. During the second phase of my third year of my PhD project, the research activity focused on the evaluation of the anti-inflammatory and antioxidant properties of *Solanum Tuberosum*. The bioaccessible peptides generated following the oral ingestion of the plant matrix determined a reduction in the enzymatic activity of iNOS and COX2 in IEC-6 cell lines in inflammatory conditions induced by LPS and INT-

γ. Finally, the evaluation of the antioxidant activity of dehydrated potato polyphenol extract was carried out using pre-column off-line DPPH.