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The health promoting potential of buffalo ricotta cheese-derived peptides

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Buffalo milk contributes to 13% of the world milk production and is abundantly produced in Southern Italy regions. Buffalo milk is appreciated for its nutritive properties and is highly suitable for the manufacturing of wide range of dairy products. Several studies showed many bioactive peptides in different dairy species such as bovine, ovine and caprine milk, but few studies have been conducted on the buffalo dairy products (1). The present work is focused on the identification of bioactive peptides released after *in vitro* simulated gastrointestinal digestion of protein fraction isolated from buffalo-milk dairy products by ultra- and nanofiltration pilot plant.

The gastrointestinal digests of protein fractions were monitored by RP-UHPLC-DAD, while, the peptide identification was carried out by UHPLC-Orbitrap-based tandem mass spectrometry. 165 peptides were identified in Yoghurt, 152 in Scamorza, 146 in Mozzarella, 136 in Grana and Ricotta and 120 in Ice Cream samples (1). The peptides belong to both buffalo caseins (α s1-, β -, k-CN) and whey proteins (α -LA, β -LG).

Six G.I. digests of dairy products were tested in a model of oxidative stress using IEC-6 cells. Among them, buffalo ricotta cheese was the most active. UHPLC-PDA-MS/MS analysis revealed the presence of two abundant β -lactoglobulin peptides (BRP: YVEELKPTPEGDL, f:60-72 and BRP2: SFNPTQL, f:168-174). To confirm the hypothesized chemical structures and study their specific biological activity, the peptides were synthesized by conventional solid-phase peptide synthesis methods. The antioxidant potential of the identified peptides was then evaluated in a model of hydrogen peroxide induced oxidative stress in IEC-6 cell line. The peptides reduce ROS release and increase nuclear factor (erythroid-derived 2)-like 2 activation and the expression of antioxidant cytoprotective factors such as heme oxygenase 1, NAD(P)H: quinone oxidoreductase 1 and superoxide dismutase (2). The bioavailability of β -lactoglobulin peptides was evaluated in intestinal transport studies through Caco-2 cell monolayer. Only BRP2 showed equal bi-directional transport and linear permeability, suggesting that it was mainly absorbed through passive diffusion. In addition to its local effects, administration of BPR2 on mice mesenteric arteries counteracts the Angiotensin II-induced vasoconstriction by Nrf2 nuclear translocation, reduction of active form of Ras-related C3 botulinum toxin substrate 1 (Rac1) and NADPH oxidase activity. The analysis at molecular level of treated vessels showed an induction of Nrf2 translocation to nucleus associated with increased expression of MnSOD and Rac1 deactivation.

The data indicate how protein fraction of buffalo ricotta cheese could be an important source of antioxidant compounds, as well as YVEELKPTPEGDL and SFNPTQL peptides could be considered as an “ingredient” for nutraceuticals formulations and functional and personalized foods, in order to prevent the onset of some gastrointestinal pathologies and cardiovascular diseases.

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