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Angiotensin-converting enzyme-inhibitory activity of extracts of hydrolysed k-casein glycomacropeptide: stability under simulated gastrointestinal digestion

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Abstract

Dietary proteins usually possess a wide range of nutritional, functional and biological properties; many of such biological properties have been attributed to physiologically active peptides, which are encrypted within the protein sequence.
k-Casein glycomacropeptide (CMP) – one of the main components of whey, is released in the first step of (enzymatic) cheese making – and has been claimed to act as mediator in important biological pathways.
Peptides released from CMP via enzymatic or microbial activity have indeed been proven to posses inhibitory activity against the angiotensin-converting enzyme (ACE). However, they will not act properly in the human body unless they can resist gastrointestinal digestion, as well as be absorbed and reach the cardiovascular system in active form. On the other hand, several studies have already demonstrated the important role of gastrointestinal digestion upon ACE-inhibitory peptide formation.
Therefore, the aim of this study was to evaluate the ACE-inhibitory activity of peptide extracts obtained via hydrolysis of CMP, using an extract of *Cynara cardunculus* as enzymatic vector. Moreover, such peptides were subjected to simulated gastrointestinal digestion, in order to assess their stability and the evolution of their ACE-inhibitory activity.
A 40 g/L solution of CMP, hydrolysed with 8.5 %(w/w) commercial crude extract of *C. cardunculus,* exhibitedACE-inhibitory activity corresponding to IC_{50} values of 296.0 mg/mL (total fraction), 63.0 mg/mL (<3000 Da fraction) and 717.0 mg/mL (>3000 Da fraction).
The identification of peptides proceeded by LC-MS, and permitted identification of the following peptides: MAIPPKKNDQD (k-CN f106-115), as potentially responsible for antihypertensive activity; and TVQVTSTAV (k-CN f161-169) and MAIPPKKNDQD (k-CN f106-115), as potentially responsible for antithrombotic activity, because both encompass sequences analogous to the bioactive fragment f400-411 of fibrinogen g-chain. A new peptide, KTEIPIN (k-CN f116-123), was also identified to relatively high concentrations, with a promising antihypertensive activity. Studies concerning the *in vitro* simulation of gastrointestinal digestion were conducted; the gastrointestinal stability of the total extract and of the <3000 Da peptide fraction, as well as of the plain CMP (used as control), following incorporation in water and fruit juice, was tested in said gastrointestinal model, but none of said fractions was significantly affected.