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Abstract

Peptides derived from food proteins show many biological activities *eg*. reduce blood pressure, prevent from thrombosis, regulate glucose level, act as antioxidants and antibacterial agents etc. Apart from biological functions, peptides affect all five taste sensations, namely: bitter, salty, sweet, sour and umami. Peptides that naturally occur in proteins and their hydrolysates are mainly bitter.

The main purpose of dissertation was the analysis of the relationship between the structure (*i.e.* amino acid sequence) of food protein originating peptides and their bitter taste by use of selected *in silico* methods. The next step was to verify the results *in vitro*. The following *in silico* methods were applied in the study: principal component analysis (PCA), multilinear regression and computer simulation of hydrolysis. Two first ones are defined as chemometric methods. The sequences of bitter peptides were implemented from BIOPEP database. The studies were carried out for 51 dipeptides, 51 tripeptides and 23 tetrapeptides.

Application of principal component analysis led to show that the following main physicochemical properties had an impact on the bitter taste of dipeptides: molecular weight, bulkiness, number of carbon and hydrogen atoms, polarity and hydrophobicity (N- and C-end). The attributes deciding about the bitter taste of tripeptides were: molecular weight, bulkiness (C-terminal and middle residue) and molecular weight, bulkiness and propensity to be exposed to solvent (N-terminal residue). In case of tetrapeptides, their bitter taste was dependent on: polarity and hydrophobicity (N-terminal residue), bulkiness and hydrophobicity (C-terminal residue). Also other variables affected the bitter taste of tetrapeptides. There were: molecular weight and bulkiness of amino acids located in position 2-3 of a tetrapeptide chain. It was observed that typical bitter dipeptide should possess N- or C-terminal isoleucine, valine and proline. Typical residues for bitter tripeptides were: proline, glycine and leucine (N- or C-end of a peptide) or „GG" motif that is known as bitter taste intensifier of tripeptide with XGG sequence. Bitter tetrapeptides should be composed of amino acids possessing ring, especially phenylalanine, proline and ones possessing hydrophobic side chains (*eg.* glycine). They should be located in position 2-3 of tetrapeptide.

Such regularities were observed by analyzing the results obtained both by use of PCA and multilinear regression. Moreover, the model „bitter taste of peptides *vs.* $R_{caf.}$" was found as the most reliable, in terms of explaining above-mentioned relationships. Multilinear regression led to distinguish di-/tri-/tetrapeptidic indicators of bitter taste (*i.e*. bitterness indicators). It was possible to obtain 17, 12 and 7 of such indicators, respectively.

From among 77 sequences of food proteins representing essential proteinogenic food sources, milk and soybean proteins were the substrates for an *in silico* hydrolysis using BIOPEP. Dozens of enzymes were applied to hydrolyze them in the combination: „one substrate (sequence):one enzyme". The highest number of peptides, including bitterness indicators were potentially released from milk and soybean proteins using: papain, bromelain, ficin and proteinase K. Obtained *in silico* hydrolysis results were the basis to hydrolyze milk and soybean proteins *in vitro* and then identify the peptides in the hydrolysate samples by means of liquid chromatography and mass spectrometry (LC-MS).

The identification of peptides derived from hydrolysates of milk and soybean proteins showed the differences between the *in silico* and *in vitro* results in terms of the number of peptides released. 38 peptides were identified in the samples of all hydrolysates: 28 sequences were found in milk protein hydrolysates, whereas 10 in soybean hydrolysates. *In silico* hydrolysis results revelaed 342 peptides (227 derived from milk protein hydrolysates and 115 originating from soybean hydrolysates). The most effective enzyme *in silico* was bromelain, whereas the least effective one was proteinase K. In laboratory conditions, they were ficin and proteinase K, respectively. The level of compatibility (comparability) of *in silico* and *in vitro* results was ~11%. Despite the differences between the results obtained in different conditions (*i.e. in silico* and *in vitro*) it can be concluded that the results of studies particularly concerning the analysis of „structure and bitter taste of peptides" are valuable for scientists and food technologists considering foods in terms of their atractiveness for consumers. The *in silico* results concerning peptide as tastants can be supportive in the sensory analysis of foods and their components, if the relevant chemometric method is selected and then used, data applied possesses good quality and prediction model is well-constructed.