UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

FACULTY OF FOOD SCIENCE

CHAIR OF FOOD BIOCHEMISTRY

Dissertation

Gouda cheese with the modified content of β-casein as the source of metabolic syndrome preventive peptides - *in silico* and *in vitro* experimental studies

mgr inż. Damir Mogut

Supervisor: Prof. dr hab. inż. Anna Iwaniak

Abstract

Definition of metabolic syndrome covers the clustering at least three of the following body dysfunctions: waist obesity, dyslipidaemia, high blood pressure and/or high glucose level. According to the literature reports, peptides acting as: dipeptidyl peptidase IV (EC 3.4.14.5) inhibitors, α-glucosidase (EC 3.2.1.20) inhibitors, angiotensin converting enzyme (EC 3.4.15.1) inhibitors, α-amylase (EC 3.2.1.1) inhibitors and antioxidative as well as cholesterol level reducing peptides may be useful food components helpful in the prevention in metabolic syndrome. The action of above-mentioned peptides is related to regulation of: blood glucose concentration, blood pressure level, maintenance the pro- and antioxidative balance and concentration of blood cholesterol.

Dairy products including ripened cheeses can be considered as functional food. It is resulted from the presence of milk proteins containing the valuable food components showing biological activities. These valuable components are the biopeptides exhibiting the above-mentioned bioactivities which act as metabolic syndrome preventive agents. It is scientifically evidenced that β-casein is found the richest source of peptides with varieties of bioactivities. Literature data also indicated the potential of ripened cheeses as the sources of biopeptides. Considering these facts, the aim of work was the analysis of Gouda cheese with modified content of β-casein as the source of anti-metabolic syndrome peptides.

The work presents the results of experiments carried out according to so-called integrated (hybrid approach). It combined the aspects of *in silico : in vitro* analyses of peptides with ACE inhibitory (i.e. cardiovascular dysfunction preventive agents), dipeptidyl peptidase IV- (DPP4) and α-glucosidase inhibitory (i.e. type 2 diabetes preventive agents) and antioxidative (anticancer preventive agents) potentially occurring in Gouda cheese with reduced, normative and increased content of β-casein.

In silico studies concerned the comparative analyses of bovine casein sequences by determining their profiles of potential biological activities (using BIOPEP-UWM database) and chemometric analyses such as principal component analysis (PCA) and multilinear regression (MLR). Chemometrics was applied to study the relationships between the structure (i.e. sequence) and DPP4 inhibitory as well as antioxidative bioactivity of dipeptides. *In vitro* experiment concerned the production of three variants of Gouda cheese (with reduced, normative and increased content of β-casein). Cheese was produced in the Chair of Dairy Science and Quality Management of Faculty of Food Science at Warmia and Mazury University in Olsztyn. Each Gouda cheese variant was subjected to produce the water soluble peptidic extracts after 1st and 60th day of cheese maturation. It gave in total 6 „variants" of

cheese samples that were aimed to assay their ACE-, DPP4-, α-glucosidase-inhibiting and antioxidative effects as well as identify the peptides responsible for above-mentioned bioactivities.

Based on *in silico* studies it was found that all casein sequences are potential sources of ACE- and DPP4-inhibiting as well as antioxidative peptides. β-Casein had the best potential to act as the source of ACE and DPP4 inhibitors whereas α_{S1}-casein was found the best source of antioxidative peptides. Chemometric analyses (i.e. PCA and MLR) revealed that „ideal" dipeptide DPP4 inhibitor should contain: Pro, Trp, His, Phe, Tyr, Ile, Leu or Val. To be an „ideal" antioxidative peptides it is recommended to contain Pro, His, Leu or Val.

In vitro studies revealed that regardless the Gouda cheese variant, all water soluble peptide extracts exhibited ACE and DPP4 inhibitory as well as antioxidative activities. None of them inhibited α-glucosidase. The highest ACE-inhibiting effect was indicated in the samples derived from Gouda cheese with normative content of β-casein (after 60th day of maturation). Gouda cheese with increased content of β-casein and 1st and 60th day of maturation displayed the highest DPP4 inhibitory activity. The highest antioxidative bioactivity (based on ABTS test) was found in the samples of water soluble peptide extracts derived from Gouda cheese with increased content of β-casein (after 60th day of maturation). DPPH test revealed the best results in terms of antioxidative bioactivity in the samples derived from cheese with increased content of β-casein (but after 1st day of maturation).

In silico identification enabled to define 232 ACE inhibitors, 201 DPP4 inhibitors and 77 antioxidative peptides in all casein sequences analysed. Based on *in vitro* i.e. LC-MS analysis it was possible to identify 81/61 of ACE inhibitors, 18/21 DPP4 inhibitors and 35/23 antioxidative peptides. These results were obtained for the samples derived from Gouda cheese with reduced content of β-casein (after 1st/60th day of maturation, respectively). In the samples derived from Gouda cheese with normative content of β-casein (after 1st and 60th day of maturation, respectively) it was possible to identify: 79/47 ACE inhibitors, 16/19 DPP4 inhibitors, and 37/19 antioxidative peptides. In the case of the samples originating from Gouda cheese with increased content of β-casein the following numbers of peptides were identified: 68/57 ACE inhibitors, 18/19 DPP4 inhibitors, and 36/25 antioxidative peptides (1st/60th day of maturation, respectively).

To summarize, based on the identification of peptides and bioactivity assays of Gouda cheese water soluble peptide extracts it is not possible to clearly conclude that Gouda cheese with increased content of β-casein is better source of metabolic syndrome preventive peptides than cheeses with reduced and/or normative content of the above-mentioned casein fraction.

The studies enabled for the clear conclusion that regardless the Gouda „cheese variant“ – they have the protective potential in metabolic syndrome dysfunctions. However, this issue requires more intensified studies including the simulation of Gouda cheese hydrolysis using digestive tract enzymes and the bioactivity evaluation of generated hydrolysates.