Epigenetics is a powerful phenomenon with wide-ranging effects on many aspects of biology, and vast potential in medicine. It describes the reversible and heritable mechanisms of gene expression regulation without changing the DNA sequence. Epigenetic regulation is a natural occurrence and can be influenced by several factors including age, diet or environment stimuli. These modifications can have damaging effects that can result in many civilization diseases such as cancers. Scientists are continuously uncovering the role of epigenetics in a variety of human disorders. One of the most broadly studied epigenetic modifications is DNA methylation. In this case, DNA is tagged with small molecules called methyl groups that stick to cytosines, thereby often modifying the function of the genes and affecting gene expression.

DNA methylation is a reversible process, thus new substances are need to stimulate the methylome. DNA methylation control has become a promising target for disease prevention strategy. Because the chemopreventive approach is addressed mainly to healthy people with an increased risk of disease, the most valuable group of prophylactic factors appear to be food ingredients. There are a variety of different methods to assess global DNA methylation, but most of them are still expensive and platform specific. Unfortunately, the equipment limitations of laboratories working in the field of food science make epigenetic studies difficult to perform. A relatively inexpensive and simple method for global DNA methylation assessment can be basic electrophoretic techniques (agarose gel, capillary) or more advanced - comet assay. The latter is widely used to detect and quantify DNA strand breaks in eukaryotic cells. Comet assay has been already used in food science to assess the genotoxicity of food components or food irradiation by detection of DNA strand breaks in single nuclei. The adaptation of the comet assay proposed by Wentzel et al. (2010) to measure the global DNA methylation level in single cell is based on utilization of the two restriction endonucleases: MspI and HpaII. These enzymes recognize the same sequence in DNA (5'-CCGG-3') but show different sensitivities in relation to DNA methylation. Thus, the higher level of DNA methylation, the larger difference in the global amount of DNA in the comet tails of HpaII digested versus MspI digested nucleoids occurs. In this project we propose to optimize basic electrophoretic methods (agarose gel, capillary) and comet assay to determine the effects of food ingredients on global DNA methylation. It is planned to select *in vitro* model that will reliably reflect conditions in the human body. Verification of methylation sensitive comet assay for determining the effect of food ingredients on global methylation will be carried out using catechins, the well known stimulators of DNA methylation. The reference method using the PCR technique (EpiMark®) will be used to verify the obtained results.

It was long thought that methylation of cytosine was the only base modification in mammal DNA considered as an epigenetic mark. Recently, hydroxylated derivative of methylocytosine (5-hmC) in DNA has been proposed as novel epigenetic mark as well. For this reason, it is planned to extend the application of comet assay to evaluate the level of 5-hmC. In proposed method, nucleoids will be firstly modified by conversion of 5-hmC to glucosylated form. Subsequently, nucleoids will be exposed on restriction endonucleases (MspI, HspaII). Glycosylation step will allow to distinguish methylated form of cytosine from its hydroxylathed derivative, because glucosylated form is indigestible by MspI. The reference method using the PCR technique (EpiMark®) will be used to verify the results obtained from proposed version of comet assay. If the reference method confirms the results obtained by comet assay, the impact of oxidative stress on the level of 5-hmC will be determined using proposed modification of comet assay. Hydroxymethyl derivative of cytosine is formed as a result of oxidation of 5-mC by oxidoreductases. Thus, it can be expected that demethylation process depends on the redox status of the cell.