

## **Impact of Lactic Acid Fermentation on Sour Cherry (*Prunus cerasus* L.) Juice: Flavonols Composition and Health Implications**

**The scientific goal of the project** is to enhance knowledge of the sour cherry flavonols and their biological activities. The main attention is focused on flavonols of the fermented sour cherry juice, primarily their aglycones, which is planned to be achieved by the fermentation process of juice using plant-associated *Lactobacillus* sp. Lactic acid bacteria are named among microorganisms capable of producing  $\beta$ -glucosidase enzyme to hydrolyze glycosides to free aglycones. The lactic fermentation process of sour cherry juice will be optimised and the characteristics of the flavonols composition will be examined using the high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS). Finally biological activities of identified flavonols will be studied using their chemical standards *in vitro* (the antioxidant, anti-inflammatory, anti-cholinesterase (anti-ChE) activities and the inhibition of amyloid  $\beta$  (1-42) aggregation) and in the animal model of larval and adult zebrafish (*Danio rerio*).

**Concept and research plan.** Bioavailability of polyphenols after oral administration is conditioned by the hydrolysis of the glycoside linkage by microbes in the small intestine. Additionally, the lactic fermentation process of sour cherry juice is crucial for the detailed characteristics of the flavonols composition. Thus the selection of bacteria strains (among plant-associated *Lactobacillus* sp.) exhibiting the highest  $\beta$ -glucosidase activity can improve the fermentation process. Then the flavonol profile and content in the obtained juice will be analyzed by HPLC-MS. An *in vivo* experiment using zebrafish larvae is planned to evaluate the impact of sour cherry flavonols on their behaviour. The Fish Embryo Acute Toxicity test will be performed to determine a range of effective concentrations and/or the lethal dose expressed by the LC50 index. The potential neuroprotective and neuronal repair effects of sour cherry flavonols will be tested in a zebrafish model of 6-OH-dopamine-induced neurodegeneration (the model of Parkinson's disease). The evaluation of above mentioned *in vitro* biological activities is an important element of the project to identify flavonols exhibiting effects crucial for the potential treatment of neurodegenerative disorders. Last but not least, 3-month-old adult zebrafish will be treated with flavonols for 30-day exposure. The obtained brains will be analysed using HPLC-MS method to confirm flavonol's bioavailability. Additionally, activity of antioxidant enzymes and cyclooxygenase -2 (COX-2), the selected inflammatory markers (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) and genes expression (iNOS, NF- $\kappa$ B, TNF- $\alpha$ ) in the adult zebrafish brain homogenate will be determined to study the flavonols impact on oxidant homeostasis and neuroinflammation.

**Significance of the research project.** Lots of scientific research is focused on improving the quality of public health due to high frequency of civilisation diseases. Excessive free-radical reactions and exacerbated inflammatory processes are named as the causes of the neurodegenerative disorders. In turn, phenolic compounds present in food are suggested to be the reason for its pro-health actions. The biological effects of sour cherry are in the limelight for some time due to its high polyphenolic contents, antioxidant effect, the ChE and COX-2 inhibition. Moreover, dietary flavonols are known bioavailable molecules with pro-health actions such as antioxidation, anti-inflammation and many others. However, we are still seeking answer to the question of the role of individual flavonols in the activities of the whole fruit. It has been well documented that bioavailability of polyphenols after oral administration is contingent on the hydrolysis of the glycoside linkage. Thus the sour cherry juice fermentation using selected bacteria *Lactobacillus* sp. is crucial for the detailed characteristics of the potential bioavailable flavonols. *Danio rerio* was selected to evaluate the *in vivo* toxicity and neurobiological activity of flavonols. Zebrafish is organism becoming an increasingly popular animal model in preclinical research. Its genome has been fully sequenced and has about 70% concordance with the human genome as well as 80% concordance for genes involved in the pathogenesis of human diseases. Last but not least, the interest in the antioxidant, anti-ChE, anti-COX-2 activities of flavonols and their capability to inhibit the amyloid  $\beta$  (1-42) aggregation is considered due to the amyloid and cholinergic hypothesis of Alzheimer's disease.

**Expected results.** The results will be a great summary of multiple biological activities of the sour cherry flavonols and not only for the fruit extract containing a number of polyphenols. This approach will also provide answer for two questions: (i) how important is the relationship between positioning of substituents in a molecule and its biological activity?; (ii) whether better health-promoting activities are shown by single flavonols or a mixture of them? As a result, we will obtain the necessary information for formulating future dietary recommendations.