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Introduction

The enzymes acetyl- (AChE) and butyrylcholinesterase (BChE) hydrolyze choline esters, including acetylcholine (ACh). As a result of cholinergic neurons losses, the concentration of AChE in the brain significantly decreases and the activity of BChE rapidly increases. The process is involved in β -amyloid protein formation and memory loss. Inhibition of AChE and BChE activity allows more effective usage of acetylcholine concentrations in brain and improves cholinergic neurotransmission. Fractionated and *in vitro* digested extracts from coffee were considered in this study as potential AChE and BChE inhibitors possibly alleviating the symptoms of Alzheimer's disease.

Materials/Methods

The digestion of fraction from coffee extracts obtained from Robusta Cherry type (*Coffea canephora* L.) and Arabica Cerrado type (*Coffea arabica* L.) roasted to various degrees (green, light and dark) was carried out in a simulated digestive system, reproducing physiological processes of the gastrointestinal tract supplemented with a mixture of selected probiotic bacteria. The evaluation of AChE and BChE inhibition was assessed for coffee extracts taken after digestion using an isothermal titration calorimetry that allowed to determine the most effective fraction of coffee extracts limiting the enzymes activity and protecting acetylcholine from the excessive degradation. The method is according Budryn et al. (2018).

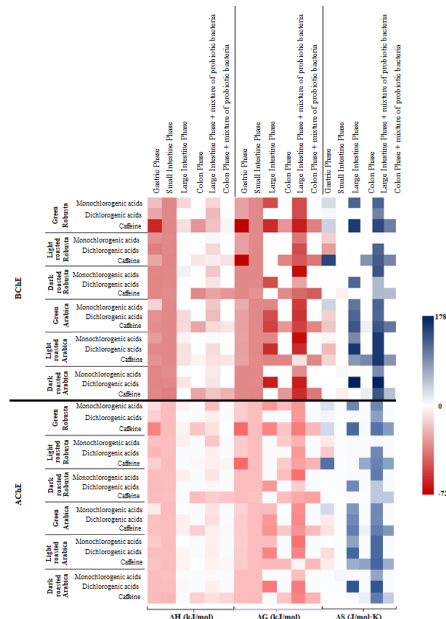


Figure 1: Interaction parameters between ChEs and coffee fraction extracts after digestion *in vitro*

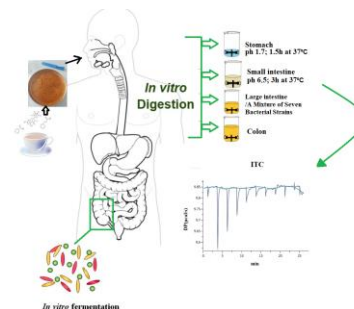


Figure 2: Obtaining of fraction of coffee extracts after *in vitro* digestion

Results/Discussion

Extracts from coffee fractions that underwent simulated *in vitro* "digestion" were combined with AChE, BChE and

acetylcholine (ACh) and formed strong complexes. Binding at the active site of enzymes was competitive and resulted in a blocked ACh hydrolysis and it increased the content of free ACh. Extract from Robusta coffee and its fractions after "digestion" at the "gastric" stage showed the highest affinity to BChE and formed the most stable complexes with the enzyme. The study also showed that the addition of bacteria to the "digestive" system increased the affinity of bioactive compounds contained in "digested" fractions of coffee extracts. This means the increased bioavailability of active compounds from coffee caused by probiotic bacteria in the "digestive" tract.

Conclusion/Perspectives

The ITC analysis of the affinity of digested coffee fractions to AChE and BChE enzymes allowed to determine the most efficient cholinesterase inhibitors. The obtained results expanded the knowledge on the possibilities of using of the coffee fractions as an alternative to drinking coffee and on the bioavailability of bioactive coffee compounds in the context of the treatment of Alzheimer's disease (project No. UMO-2018/29/N/NZ9/01160).

References:

- Budryna et al. Evaluation of butyrylcholinesterase inhibitory activity by chlorogenic acids and coffee extracts assed in ITC and docking simulation models. *Food Res Int.* 2018;108:268-277.DOI:10.1038/s41598-017-18800-1
 Bukowska et al. Acetyl- and butyrylcholinesterase - structure, functions and their inhibitors. *Current Topics in Biophysics* 2007, vol. 30 (suppl. A), 11-23