Summary of the publication cycle entitled:

"Interactions of bioactive ingredients of coffee and selected functional supplements as factor modifying their potential biological activity"

Consumption of foods and beverages with high levels of phenolic compounds is associated with the prevention of coronary heart disease, cancer and other, so-called civilization diseases. In many of these disorders, oxidative stress is involved, that results from the overproduction of reactive oxygen species (ROS), which are also produced due to pro-oxidative enzymes activity, including lipoxygenase (LOX) and xanthine oxidase (XO). The human body uses its own enzyme system and egzogenous antioxidants, e.g. phenolic compounds to defense against ROS. Antioxidant efficacy of phenolics results in multidirectional action: they may inhibit free radical reactions by inhibiting the formation of lipid radicals, interrupting the autoxidase chain reaction, suppressing singlet oxygen, acting as reducing agents to convert hydroperoxides to stable compounds as chelating agents of transition metal and as inhibitors of prooxidative enzymes.

Epidemiological data indicate a significant correlation between regular coffee drinking and a reduced risk of type 2 diabetes, hepatocellular carcinoma, endometrial carcinoma, and colorectal cancer. These benefits can be partly attributed to chlorogenic acids. Spices and herbs are added to foods since ancient times not only as a flavoring agents but also as medicines and food preservatives. The health benefits of spices include, but are not limited to, antithrombotic, anti-atherogenic, hypolipogenic, hypoglycemic, hypotensive, anti-inflammatory, anti-arthritic and ability to thrombocyte aggregation inhibition. In this work, cinnamon, ginger, cardamom and chili were used to enhance the infusion of roasted coffee, which affects not only the taste and aroma, but also the antioxidant properties of the coffee beverage.

The main determinants of biological activity of polyphenolic compounds derived from food are their metabolism and bioaccessibility, and the very often overlooked issue in food research is the interaction of its components. The aim of this study was to determine the polyphenol profiles of roasted coffee and selected functional additives and the determination of the type of interaction of biologically active compounds in terms of their bioaccessibility. The results obtained were compared with the effects in the model system to determine the influence of the food matrix on the type of interaction observed within the biological activity.

UPLC/MS analyses allowed the identification of several bioactive coffee compounds in coffee infusions. These were mainly substances of the hydroxycinnamic acids group (Durak et al., **I.-III**.). In the work Durak et al. **III**, the phenolics bioaccessibility factor (APC) was

determined. Its value was 0.88, which may indicate the relatively good efficiency of the extraction of the active compounds by the simulated digestive system. We have identified 8 phenolic compounds from cinnamon infusions (Durak et al. **I.)**: four compounds from the proanthocyanidin class of flavonoids, cinnamic acid as a representative of the group of hydroxycinnamic acids and other polyphenols such as: coumarin, (Z)-cinnamaldehyde, (E)-cinnamaldehyde. In ginger infusion 5 volatile compounds have been identified: these are gingerol derivatives. In addition, all these compounds were present in ginger extracts after digestion (Durak et al., **II.**) and in the aqueous extract of cardamom, protocatechuic acid, vanillic acid, ρ-coumaric acid and ferulic acid were identified (Durak et al., **III.**). Qualitative and quantitative analysis of the phenolic profile of chili showed the presence of eight biologically active compounds. Capsaicin has been shown to be the dominant active compound present in the dried red chili peppers at the highest concentration, but the presence of quercetin, apigenin and luteolin derivatives has also been demonstrated (Durak et al., **IV.**). The total concentration of phenolic compounds in the chili extract (966.27 ± 14.46 mg / g) was much higher than in case of coffee extract (224.9 ± 11.25 mg / g), and extracts the examined spices such as cinnamon (42.6 ± 2.11 mg / g) (Durak et al. **I**.), ginger (5.4 ± 0.01 mg / g) (Durak et al., **II.**) and the cardamom (2,121 ± 0,05 mg / g) (Durak et al., **III**.). Taking into account the polyphenol profile of tested spices the following order was obtained: chili> cinnamon> ginger> cardamom. Despite the very low concentration of active compounds, their high bioaccessibility for cardamom extract was noted (APC = 2.85) (Durak et al., **III**.).

The antioxidant properties of coffee extracts and the extracts of proposed spices were tested using several methods based on different mechanisms of action. The results are presented as the EC50 (mg / mL), the concentration of sample necessary to produce 50% of the tested activity (Durak et al. **I.-IV**.). In the case of coffee extract, antioxidant potential (expressed as the ability to neutralize the ABTS $^{\bullet+}$ radical) was 1.86 mg / mL. In terms of antioxidant capacity aromatic additives can be ranked as follows: cinnamon (1.52 mg / mL)> cardamom (3,68 mg / mL)> ginger (6.40 mg / mL)> chili (16.11 mg / mL). After *in vitro* digestion, the decrease in EC50 values has been observed. For coffee digested extract EC50 decreased to 1,47mg / mL and results for digested spice extracts can be arranged in the following order: cinnamon (1.18 mg / mL)> card (2.24 mg / mL)> ginger (3, 82 mg / mL)> chili (6.59 mg / mL). Cinnamon has been found to be the source of compounds with the highest antioxidant activity against the ABTS $^{\bullet+}$ radical. The second study was the assessment of the reducing power (Durak et al., **III.,** Durak et al., **IV.**). In this case, EC50 for the aqueous coffee extract was 0.58 mg / mL, and

interestingly, this ratio increased to 1.21 mg / mL after *in vitro* digestion. Chili showed better properties than the cardamom, although in both cases the reducing power increased to a similar value (4.94 mg / mL for chili and 5.07 mg / mL for cardamom) after the process of the simulated digestion. Taking into consideration the ability to chelate transition metal ions (Durak et al., **III.,** Durak et al., **IV.**), the best results were noted for the infusion of chilli, wherein the process of digestion has caused the increase in the activity of this extract, as opposed to coffee extracts that chelation capacity after the process of *in vitro* digestion strongly decreased (from 0.58 mg / mL to 1.21 mg / mL). Cardamom has been found to be a source of compounds with very low ability to chelate transition metal ions, although the properties of this extract slightly increased due to *in vitro* digestion. In the case of the ability to neutralize the hydroxyl radical (Durak et al., **III.,** Durak et al., **IV.**) there was a significant decrease in EC50 values in extracts from coffee and cardamom as a result of simulated digestion. Taking into account the chilli extract, the activity of the infusion was similar to the activity of the coffee extract, but after the *in vitro* digestion process, a significant decrease in this activity was observed. This process also has resulted in a significant decrease in the ability to neutralize the superoxide radical by compounds contained in coffee infusions (EC50 14.96 mg / mL and 10.30 mg / mL respectively).

The next stage of the study was estimation of interactions between active compounds of coffee and the proposed aromatic additives. The results were compared to the interactions of pure chemical standards forming the model systems. Considering the ability to scavenge the $ABTS^{\bullet+}$ radicals, an antagonistic reaction was observed for a mixture of coffee and cinnamon extracts and in a model system composed of chlorogenic and cinnamic acid (Durak et al., **I.**). In case of coffee with ginger, water infusions acted synergistically, in the extracts after simulated digestion the additive reaction was observed, and antagonism in the model system (Durak et al., **II.**). Water infusions of coffee and cardamom acted synergistically, and in the case of extracts after simulated digestion and in the model system, the antagonistic reaction was observed (Durak et al., **III.**). The bioactive compounds contained in coffee and in chili spice acted synergistically, although an antagonism was noted after *in vitro* digestion (Durak et al., **IV.**). Analyzing the reducing power, synergism was observed in the case of aqueous and digested coffee and cardamom extracts and in the model system created for them (Durak et al., **III.**). The same type of interaction was noted for coffee and chilli extracts before and after digestion (Durak et al., **IV.**). Compounds capable of chelating transition metal ions contained in coffee and cardamom extracts acted synergistically, but after the simulated digestion process

and in the model system, an additive reaction was observed (Durak et al., **III.**). Coffee and chilli extracts acted antagonistically and the *in vitro* digestion did not affect the type of observed interaction (Durak et al., **IV.**). In turn, in the case of the potential for OH radical neutralization a strong antagonism was observed between the compounds contained in coffee and cardamom infusions, the additive reaction after *in vitro* digestion, and the synergism of chlorogenic and vanillinic acid in the model system (Durak et al., **III.**). Coffee and chilli ingredients acted synergistically to the hydroxyl radical neutralization, although antagonism has been reported for extracts after *in vitro* digestion (Durak et al., **IV.**). Coffee and chilli ingredients have been synergistic hydroxyl radical scavengers, although antagonism has been reported after digestion *in vitro* (Durak et al., **IV.**). Compounds capable of neutralizing the superoxide radical ($O_2^{\bullet -}$) present in the coffee and chili infusions acted synergistically (Durak et al. **IV.**). The same relationship was observed for coffee with cardamom, but for these extracts after digestion and in the model system, strong antagonism was observed (Durak et al., **III.**).

Considering the interactions within the lipoxygenase (LOX) activity inhibition, the synergism of LOX inhibitors contained in coffee and cinnamon infusions and in the model system was observed, however antagonism was observed for extracts after the simulated digestion (Durak et al., **I.**). In case of coffee with ginger, the infusions acted antagonistically, but for extracts after *in vitro* digestion and in the model system, a synergistic reaction was noted (Durak et al., **II.**). By analyzing the interactions of coffee and cardamom infusions and their model system synergism was demonstrated, however the digested extracts acted antagonistically (Durak et al., **III.**). Taking into account the ability to xanthine oxidase (XO) inhibition, for both digested and non-digested extracts of coffee and cardamom synergistic reaction was observed and also in the model systems composed of chlorogenic and vanillic acid (Durak et al., **III.**).

In conclusion, our findings have shown the multidirectional biological activity of the proposed raw materials, which justifies their potential use as functional additives. Particularly valuable are their properties enabling the prevention/support of the treatment of civilization diseases associated with excessive production of free radicals, including the ability to inhibit the activity of pro-oxidative enzymes.

References:

I. Durak A., Gawlik-Dziki, U., Pecio, Ł. 2014. Coffee with cinnamon – impact of phytochemicals interactions on antioxidant and anti-inflammatory in vitro activity. Food Chemistry, 162, 81-88

II. Durak A., Gawlik-Dziki, U., Kowalska, I., 2014. Coffee with ginger- interactions of biologically active phytochemicals in the model system. Food Chemistry, Vol. 166, 261-269.

III. Durak A., Gawlik-Dziki, U., Kowalska, I., 2016. Evaluation of interactions between coffee and cardamom, their type, and strength in relation to interactions in a model system, CyTA - Journal of Food, Vol. 15, Issue 2, 266-276. DOI: 10.1080/19476337.2016.1247298.

IV. Durak A., Kowalska, I., Gawlik-Dziki, U., 2017. UPLC–MS method for determination of phenolic compounds in chili as a coffee supplement and their impact of phytochemicals interactions on antioxidant activity *in vitro*. Acta Chromatographica. To link to this article: http://www.akademiai.com/doi/abs/10.1556/1326.2016.00173