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BOOK OF ABSTRACTS



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reared on two different substrates for more than 20 years. For that purpose, five food items were offered. Further, it was examined whether sex and social environment influenced food choice in these two strains.

METHOD / DESIGN:

D. melanogaster strains used in this experiment were maintained for more than 450 generations on two different substrates, standard cornmeal substrate and substrate modified by adding apple. Transparent plastic boxes, dimensions: 220 × 140 × 90mm, which contained five Petri dishes (r = 30mm) were used for monitoring food choice. Petri dishes were filled with five different substrates: standard cornmeal substrate and substrates that contain tomato, banana, carrot and apple. Flies were starved for 18h before being placed into each box. Virgin females and males, 3 - 5 days old, were separated and tested individually and in groups of five individuals, and foraging flies were sampled every 3 min for 1h. Four-way ANOVA was applied in order to determine difference in time that flies spent on different diets, between individuals and groups, and between sexes.

RESULTS:

Results pointed out significant differences in the time that flies spent by occupying different food items. On the other hand, sex, strain and social environment revealed no significant influence on *D. melanogaster* food choice. However, significant interaction between strain and food choice was observed. In both strains, the preference toward standard cornmeal substrate was noticed. Even more, flies reared on apple substrate spent significantly more time on Petri dish filled with standard cornmeal diet, compared to flies reared on standard substrate.

CONCLUSIONS:

These results indicated that flies chose nutritionally richer food (standard cornmeal substrate, rich in sugar and yeast), especially if they were reared on poor diet (the apple substrate). According to data from our previous surveys, substrates that we offered to flies differ in protein content and in the proportion of protein relative to the total content of organic carbon (C/N ratio), which accurately reflected the protein/carbohydrate ratio. Contrary to standard substrate, apple substrate contained smaller amount of protein and higher C/N ratio. The fact that food choice was not influenced by sex or social environment might suggest the same nutritional requirements for the best available food in both sexes, regardless of whether flies were tested individually or in a group.

T1-P-6 Proline-based deep eutectic solvents as greener alternative for obtaining polyphenol rich extracts of *Satureja Kitaibelii*

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KEYWORDS: DES; cytotoxicity; extraction; caffeic acid oligomers; *Satureja L.*

INTRODUCTION:

Aerial parts of *Satureja kitaibelii* Wierzb. ex Heuff. (Lamiaceae), in Serbia known as Rtanj's tea, are traditionally used to treat

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various respiratory, urinary and other health disorders. Extracts of this herb exhibit a significant bioactivity as well³⁷. Using deep eutectic solvents (DESs) for extraction of certain phenolic compounds is in line with the principles of green chemistry³⁸. However, the toxicity of DESs must be considered³⁹.

OBJECTIVES:

The main objectives of this work are to assess the polyphenol-extracting ability of proline (Pro) and sugar/sugar alcohol based natural DESs from commercially available Rtanj's tea, as well as to evaluate cytotoxicity of these solvents against AsPC-1 cells.

METHOD / DESIGN:

Accurately weighed Pro and sugar/sugar alcohol, were dissolved in water, frozen, and freeze-dried. The obtained seven DESs (i.e. Pro with: glucose 1:1 and 5:3, fructose 1:1 and 5:3, sorbitol 1:2, and sucrose 2:1 and 3:2) were mixed with water (30%, *m/m*). Polyphenol extraction was examined using commercial sample of Rtanj's tea (manufacturer Bojan Radosavljević, Boljevac). Powdered herb (particle diameter 100-200 μm) was extracted by sonication during 30 min at room temperature with the obtained aqueous DESs (herb-to-solvent ratio 1:20), as well as with water, absolute ethanol, or 50% (*v/v*) ethanol under the same conditions. The qualitative analysis of extracts was conducted by both HPLC and LC-MS. The content of the identified marker compounds in the extracts, i.e. rosmarinic acid (RA) and clinopodic acid O (CAO), was determined by external calibration using RA as the standard compound. Cytotoxicity of the aqueous DESs against human pancreatic adenocarcinoma cells AsPC-1 was tested at four concentration levels (5-25%), after 48 h of incubation and propidium iodide staining. The analysis on Guava® easyCyte 12HT Benchtop flow microcapillary cytometer, was performed afterwards, using InCyte® software package.

RESULTS:

Upon freeze-drying, mixtures had glassy appearance and transformed into liquids after mild heating. All obtained DESs were highly viscous, and therefore mixed with water. Qualitative LC-MS analysis of 50% ethanol extract revealed the presence of phenolic acids, flavonoids, and jasmonic acid derivatives. Among phenolic acids, the dominant compounds were caffeic acid oligomers RA and CAO. Among conventional solvents, 50% ethanol was better extracting agent than absolute ethanol or water for both RA (88.2 $\mu\text{g/mL}$) and CAO (116.8 $\mu\text{g/mL}$). Water extract was also abundant with CAO (106.7 $\mu\text{g/mL}$), but contained moderate amount of RA (21.7 $\mu\text{g/mL}$). It is noteworthy to mention that the extraction with ethanol resulted in very low yield of both phenolics, with CAO concentration, being even below detection limit in the absolute ethanol extract. Concentration of RA in the tested DES extracts was higher than corresponding one in the water extract, but lower than in the 50% ethanol extract, and varied in the range from 61.6 $\mu\text{g/mL}$ (in Pro-fructose 1:1 extract) to 85.6 $\mu\text{g/mL}$ (Pro-glucose 1:1 extract). The extraction of CAO, with six out of seven aqueous DESs, was more efficient than with 50% ethanol, resulting in CAO concentration range from 119.6 $\mu\text{g/mL}$, in Pro-sucrose 3:2 extract, to 172.4 $\mu\text{g/mL}$, in Pro-glucose 1:1 extract. Pro-fructose 1:1 extract had the lowest content of CAO (86.7 $\mu\text{g/mL}$) among the tested DESs. At the lowest tested concentration (5%), aqueous DESs did not significantly affect survival of AsPC-1 cells in comparison to the untreated cells (83.1-90.0% and 86.6% of cells remained viable, respectively). Both proline-glucose DESs demonstrated the lowest toxicity. However, at the highest concentration (25%) all aqueous DESs caused death of more than 70% of AsPC-1 cells.

37 Gopčević et al., 2019. *Plant Foods Hum Nutr* 74:179.

38 Jakovljević et al., 2020. *Plants* 9(2): 153.

39 Hayyan et al., 2013. *Chemosphere* 90(7): 2193.

CONCLUSIONS:

The obtained results indicate that proline and sugar/sugar alcohol based deep eutectic solvents are good extracting agents for phenolic compounds, especially for higher caffeic acid oligomers such as clinopodic acid O. Additionally, low cytotoxicity of tested DESs is a good starting predictor of their safety and potential usage.

T1-P-7 Assessment of litter decomposition in temperate deciduous forest: A case study in Fruška Gora, Serbia

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KEYWORDS: TeaComposition initiative; litter decomposition; litter quality

INTRODUCTION:

Our study has been conducted as a part of the large scale decomposition experiment within the global collaborative network "TeaComposition initiative". The aim of this initiative was to estimate short- to long-term plant litter decomposition rates by using standard protocols and substrates—commercially available Green tea and Rooibos tea with different decomposition rates—for comparison of the litter mass loss at numerous sites across various ecosystems worldwide.

OBJECTIVES:

The aim of our study was to test the effects of both litter type and land-use on litter decomposition in 3, 12, 24 and 36 months of incubation, by comparing the percentages of the tea mass lost.

METHOD / DESIGN:

The TeaComposition method (modified Tea bag method) involves measuring a tea bag before and after incubation in the field, and using the difference in weight as a measure of the organic material decomposed. The three localities chosen for our experiment corresponded to the three levels of protection regime established for the National park Fruška gora, with different management and treatments within the temperate deciduous forest. The guidelines of the standardized protocol of the "TeaComposition initiative" were followed throughout. Two homogenous plots were selected at each of the three sub-sites; two replicates of the two tea types were buried in the topsoil layer in each of the two blocks, resulting in four bags of each tea type per sub-site and sampling time.

RESULTS:

The values of the tea mass lost during all four incubation periods were higher for the Green tea than for the Rooibos tea. This pattern was expected because of the faster decomposition rate of Green tea due to higher content of non-lignified cellulose and of water-soluble compounds. Furthermore, the difference of the two tea types' mass loss was the highest in three-months incubation. Our study has also shown no clear pattern regarding the values variation of the tea mass loss among three different plots; however, the highest level of variation was found for the Green tea in the longer incubation periods (24 and 36 months).

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