counting the flies which were present in the upper most quadrant. The experiment was conducted for 30 min which thus completes 15 cycles. For each experiment 50 flies were taken. The experiments were carried out before noon. All the experiments were carried out in three sets. Males and females were considered separately for the experiment.

Table 1. Showing number of flies in different divisions of the tube.

Number of rotations	Division 4	Division 3	Division 2	Division 1 Uppermost
1		1	2	47
2				50
3	4		2	44
4	3		2	45
5			1	49
6	2		2	46
7		1		49
8	1		1	48
9		1		49
10	1		1	48
11		1		49
12	1	1	2	46
13	3		2	45
14	2	2		46
15	2	1		47

Observations

Flies that are placed at the bottom of the tube tend to move up due to negative geotaxis. Repetitive stimulation of 15 cycles placed the flies in the bottom of the tube and evoked the flies to climb repeatedly. The time taken for one session of training was 30 minutes following which the flies rested. Climbing index was calculated by counting the number of flies manually (Table 1).

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Antioxidant properties of fruit/vegetable substrates suitable for culturing *Drosophila melanogaster*.

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In year 2000, we established four *Drosophila melanogaster* strains reared on substrates cooked with fruits and vegetables (banana - B strain; apple - A strain; T - tomato strain; and C- carrot strain), which represent resources used for feeding in natural conditions (Shorrocks, 1972). After culturing flies for more than 60 generations,we have published those recipes as suitable for maintaining *D. melanogaster,* as well as species with similar nutritional requirements (Kekić and Pavković-Lučić, 2003). Later, we have used those strains in experiments devoted to the influence of nutrition on morphological characteristics, cuticular chemistry, life-history, and behavioral traits (Pavković-Lučić and Kekić, 2010; Trajković *et al.*, 2013; Pavković-Lučić *et al.*, 2016; Trajković *et al.*, 2017a, b).

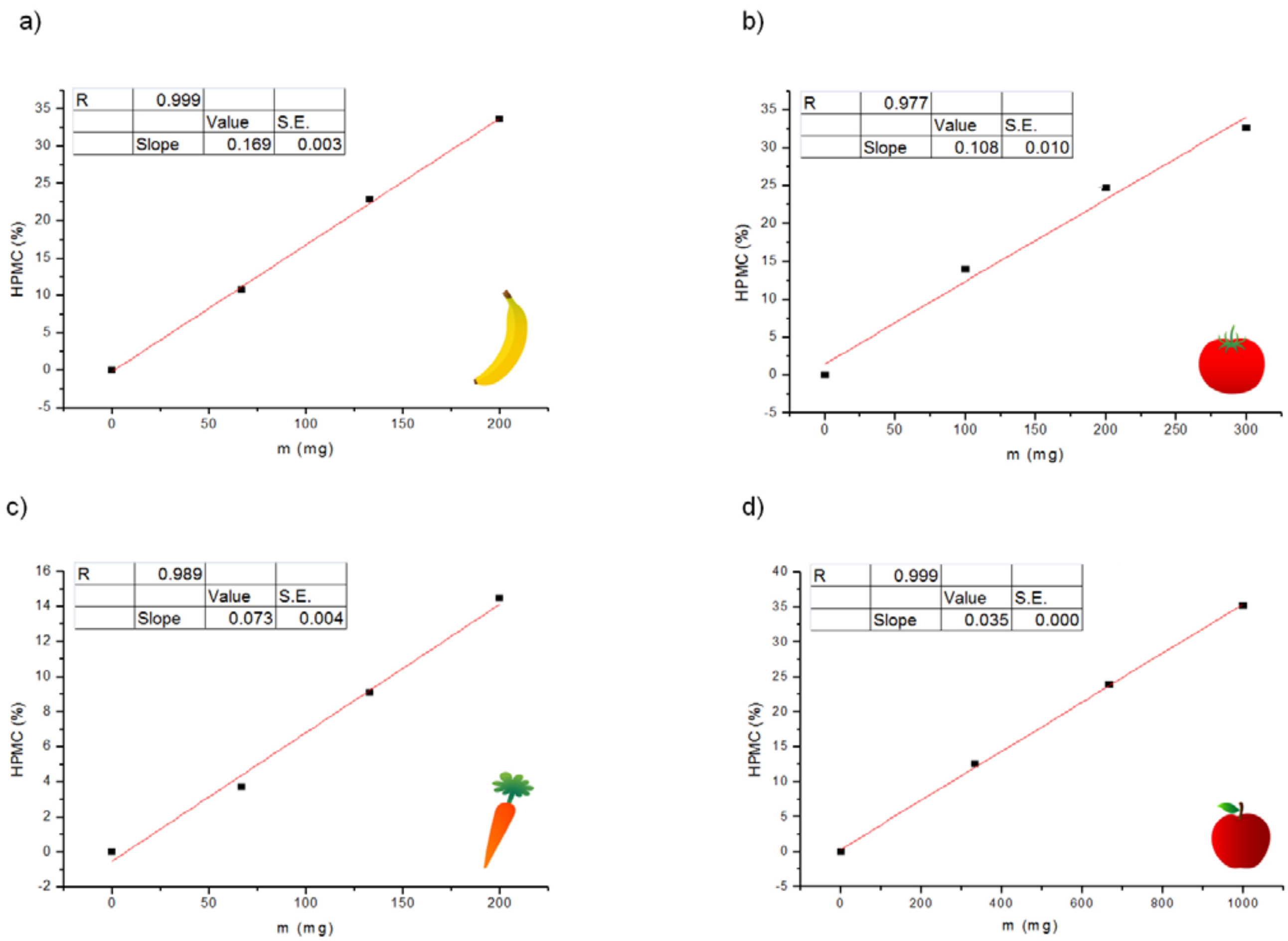

Figure 1 (a-d). Antioxidant properties of four substrates used for *D. melanogaster* culturing: banana substrate (a), tomato substrate (b), carrot substrate (c), and apple substrate (d), measured by HPMC assay.

Chemical analysis of the aforementioned food revealed differences in their protein content and C/N ratio (Trajković *et al.*, 2017a). Since fruits and vegetables are sources of phytochemicals which act as antioxidants (Slavin and Lloyd, 2012), we have recently tested antioxidant properties of the diets using an electrochemical assay based on direct current polarography. The polarographic assay applied was selected as fast, reliable, and low cost alternative to spectrophotometric antioxidant assays commonly applied in analysis of food and biological samples. Moreover, polarographic monitoring of HPMC current decrease allowed colored and turbid samples, usually unacceptable in spectrophotometric assays. Polarographic i-E curves were obtained using the Princeton Applied Research 174 Polarographic analyzer and recorded on Houston Instrument Omnigraphic 2000 X-Y recorder. The dropping time of working dropping mercury electrode (DME) (with capillary characteristics of m = 2.5 mgs^{-1} at mercury reservoir height of 75 cm) was programmed on time 1 s, while current oscillations were damped with low pass filter of instrument set at 3 s. Saturated calomel electrode (SCE) was used as reference and platinum foil as auxiliary electrode. The initial potentials were 0.10 V *vs* SCE. Potential scan rate was 10 mV/s. Clark & Lubs (CL) buffer of pH 9.8, used as supporting electrolyte, and initial solution for HPMC assay were obtained as described previously by Gorjanović *et al.* (2013a). Before each i-E curve recording, initial solution as well as solution after addition of aliquots of each analyzed samples was deaerated using pure gaseous nitrogen. Inert atmosphere was kept in the cell during each i-E curve recording. Decrease of anodic limiting current originating from HydroxoPerhydroxoMercury(II) complex [Hg(O2H)(OH)] formation in alkaline solutions of hydrogen peroxide at potential of mercury dissolution upon gradual addition of antioxidants was observed. The assay based on that decrease was optimized (Sužnjević *et al*., 2011) and applied on wide variety of food and biological samples (Gorjanović *et al*., 2013b). Dependence of decrease of HPMC anodic limiting current on volume or mass of gradually added complex samples has been followed and plotted. The slope of the starting linear part of that plot was considered as a measure of antioxidant (AO) activity. The activity was expressed as percentage of anodic limiting current decrease per volume or mass of complex samples added (%/mL or %/mg).

Based on the results, fruit/vegetable substrates could be arranged according to their antioxidant properties, from the highest to the lowest, in the following way: banana (0.169 ± 0.003%/mg) > tomato (0.108 ± 0.001%/mg) > carrot (0.073 ± 0.004%/mg) > apple (0.035 ± 0.001%/mg). We must emphasize that we always peel an apple before cooking the substrate. This could be an explanation for its low antioxidant activity, since the majority of compounds with high antioxidant potentials are found in the apple skin (Wolfe *et al*., 2003). Further, cooking destroys part of the AOs, so our results can not be compared to those obtained for fresh fruit. Nevertheless, information considering differences in antioxidant properties of aforementioned diets could be useful for further experimental purposes.

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Creation and standardization of methods for ethological analysis of *Drosophila melanogaster*: preference test and immobilization stress.

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Introduction

The many fundamental biological mechanisms conserved between *Drosophila* and *Homo sapiens* justify the study of *Drosophila* in many biological fields, including neurobiology of learning, memory, and stress (Jennings, 2011). As observed in different vertebrates, like *Rattus norvegicus*, *Mus musculus*, or *Danio*