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Bioassay of oxidative properties and toxic side effects of apple juice

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Abstract:

Introduction. Apple juice owes its beneficial properties to various biologically active compounds, e.g. antioxidants. Therefore, food science needs effective methods that would cover all the mechanisms of their effect on human metabolism. However, fruit juice production raises certain safety issues that are associated not only with production risks, but also with some natural components in the raw material. The *Allium* cepa test seems to be an effective solution to the problem. This plant bioassay has a good correlation tested on mammalian cell cultures.

Study objects and methods. Onion roots (*A. cepa)* were treated with aqueous solutions of juices and sorbic acid to assess their antioxidant profile. The toxic effects on root tissues were described according to biomass growth, malondialdehyde (MDA) concentration, and proliferative and cytogenetic disorders.

Results and discussion. The study revealed the optimal conditions for the *A. cepa* assay of the antioxidant properties of apple juice. The antioxidant activity was at its highest when the juice was diluted with water 1:9 and the onion roots were treated with sorbic acid. The lipid oxidation of the *A. cepa* roots decreased by 43%. A comparative analysis of three different juice brands showed that the difference in their antioxidant profiles was ≤ 3%. As for toxic side effects, the chromosome aberrations increased by six times in all samples.

Conclusion. The research offers a new *in vivo* method for determining the antioxidant profile of apple juice. Three juice brands proved to have irreversible cytotoxic and genotoxic effects.

Keywords: Apple juice, bioassay, antioxidant activity, side effects, *Allium cepa* test, biologically active substances

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INTRODUCTION

Apple juice is one of the most popular fruit juices in Russia. Therefore, domestic food industry needs reliable methods for its nutritional value and risk assessment. The beneficial properties of apple juice are associated with various biologically active compounds. Recent antioxidant studies show that apple juice is rich in such antioxidants as polyphenols, e.g. quercetin, phloretin, chlorogenic acid, and epicatechin. A fruit and vegetable diet reduces oxidative stress, thus preventing chronic diseases and slowing down aging. Apples and apple products are known to reduce the risk of cancer, cardiovascular diseases, asthma, and type II diabetes [1]. The chemical composition of juices depends on the variety of apples, their ripeness, climate, cultivation method, etc. Apple juice production involves a wide variety of apple cultivars but gives preference to winter and autumn varieties because they are juicy, firm-fleshed, and rich in aromatic and phenolic substances.

Consumers see apple juice as a source of biologically active compounds that are beneficial to human health. As a result, the volume of its industrial production keeps increasing. Food processing determines the nutritional value of the finished product [2]. Crushing, heat treatment, fermentation, and clarification of apples affect the phytochemical composition of apple juice. These processes decrease the amount of phenolic compounds. After heat treatment and direct extraction, fruit juice had 10% of the antioxidant properties of fresh fruits. After pulp fermentation, this figure was 3%. Pulp fermentation decreased the content of phloridzin,

chlorogenic acid, and catechin by 31, 44, and 58%, respectively. Most of the active compounds remained in apple pomace [3].

Another study compared polyphenols in apple juice after heat and high pressure treatments [4]. The phenolic profile of the resulting apple juice changed significantly. The epicatechin concentration was 0.42 mg/100 mL in the raw juice; it decreased to 0.31 mg/100 mL at 25°C and increased to 0.39 mg/mL at 65°C. Heat treatment increased the amount of catechin and chlorogenic acid, while pressure treatment decreased the amount of polyphenols. The authors linked this phenomenon to structural destruction because the rapid release of carbon dioxide led to pressure gradient.

Various plant assays of antioxidants properties receive more and more scientific attention each year. Unfortunately, different antioxidant tests use different terms and measurements [5]. Moreover, antioxidants may respond differently to different radicals or their sources. Phytochemical compounds are present in numerous products and possess numerous mechanisms of action on metabolic processes. Thus, the food industry has a wide choice of adequate antioxidant assessment methods [6]. Therefore, an objective analysis of data on bioactive compounds needs specifically tailored markers. Finally, the bioactivity of plant food products depends on a whole complex of phytochemical compounds. Lipid peroxidation is measured by the levels of malondialdehyde (MDA), β-carotene, and diene conjugates [6].

Other methods determine the total antioxidant potential according to the concentration of free radicals, e.g. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2-diphenyl-1-picrylhydrazy[l] (DPPH) assay, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, ferric reducing/antioxidant power (FRAP) assay, ferrous oxidation-xylenol orange (FOX) assay, ferric thiocyanate (FTC) assay, and aldehyde/carboxylic acid (ACA), etc.

These approaches make it possible to analyze the level of antioxidant activity both in food products and in living organisms after consumption. However, bioassays seem to be the most informative and accurate methods, since all nutritionally valuable substances are bioavailable and bioactive. Testing food matrices on laboratory animals or human cell lines is expensive and labor-consuming. Therefore, plant assays are more preferable.

Scientists compared the level of lipid peroxidation in onion roots after their treatment with apple juice and a model aqueous solution of fructose, glucose, sucrose, D-sorbitol, and malic acid. After incubation, the content of MDA in root tissues was 1.7 times higher in the model solution than in the apple juice [7]. Such results proved that the juice possessed some antioxidant activity, which lowered the carbohydrate-induced lipid oxidation almost to the control values, i.e. those of water.

Domestic regulations ban synthetic additives from juice production. Unfortunately, these measures fail to eliminate juice-related safety risks. Therefore, food producers have to check raw materials for various contaminants, such as heavy metals, pesticides, and herbicides, as well as to monitor the safety of technological production means, e.g. detergents, lubricants, packaging material, etc. Moreover, technological methods of juice processing require exposure to high temperatures during pasteurization, sterilization, etc., which can result in accumulation of toxic compounds and adducts. For example, some phytochemical compounds of plant products are known to react with cellular macromolecules during storage, thus causing cellular toxicity or even genotoxicity if they react with DNA [7, 8].

Almost all higher plants contain such natural mutagens as pyrolizidine alkaloids and some flavonoids [9]. In fact, recent studies linked the consumption of fruits and juices to cancer and asthma in children [10–13]. Finally, juices are rich in carbohydrates, and fructose and sucrose produce adverse metabolic effects on human health [14, 15]. Food scientists have developed numerous physicochemical assay methods for these toxic agents. However, bioassays seem to be the only method that gives an integrated assessment of their synergetic effect.

In this regard, the *Allium cepa* test is especially promising. This test is recommended by WHO experts as a standard for cytogenetic monitoring. The *A. cepa* assay is a popular method to define the bioindicator of cyto- and genotoxicity of xenobiotics in food products and their components [16]. The *A. cepa* test provides a prompt comparative analysis of individual compounds and their combinations. *A. cepa* cells share metabolic mechanisms with all eukaryotes, but unlike animal and human cell lines, they are not subject to transformation and can be useful in detoxification modeling. This test can screen biomarkers that determine the negative potential of food matrix toxicants for metabolic processes in onion root tissues [17].

Taking into account these indicators and the data on antioxidant activity, plant bioassays can logically be applied to various brands of apple juice [7]. However, research databases seem to contain no publications on the *Allium*-based comparative evaluation of various domestic brands of apple juice. The present research objective was to compare the antioxidant activity, cytotoxicity, and genotoxicity of various domestic apple juice brands.

STUDY OBJECTS AND METHODS

Preparation of bioassay solutions. The research featured samples of processed and clarified apple juices from four producers. The juices were purchased from a retail chain and marked as A, B, C, and D. The juices were within the expiration date, with intact packaging. The juices were diluted with bottled water in ratios 1:5, 1:9, and 1:20. Sorbic acid (Thermo Fisher Scientific, USA) simulated oxidative stress. Solutions of sorbic acid (100 and 50 mg/L) included bottled water and

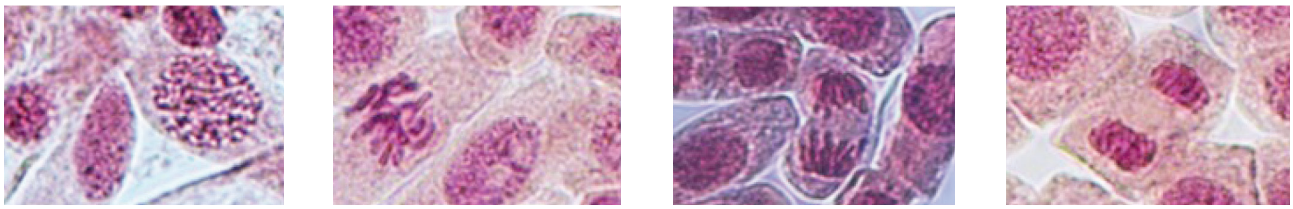


Figure 1 Mitosis phases, from left to right: prophase, metaphase, anaphase, and telophase

were prepared in a water-bath by heating to 78°C with constant stirring.

Bioassay. The bioassay featured peeled onion bulbs of the same weight (5–7 g) and diameter (≥ 3 cm). The onions were placed in 2-mL test tubes with bottled water and left for two or three days, depending on the experimental conditions, in a thermostat (24 ± 1°C) in total darkness. After two days of preliminary germination, the onions with a root length of ≥ 1 cm were placed in experimental solutions with apple juice, sorbic acid, or their mix. They were incubated in the thermostat for the next 24 or 48 h. Bottled water was used as a negative control. Ten onions were selected from each group of experimental and control samples. After preliminary three days of germination and two days of treatment with solutions of different juices, some onions were thoroughly washed and then incubated in bottled water for another 48 h at 25 °C to be tested for recovery treatment. After the experiment, all roots were cut off, dried with filter paper, and weighed. The weight gain was determined as the arithmetic mean for each solution.

Staining and microscopy. A 2% solution of acetoorcein was used to stain the preparations of onion apical root cells. The solution included 1 g of orcein dye per 50 mL of 45% acetic acid. A 70% solution of ethyl alcohol facilitated the long-term storage in the refrigerator. The experiment involved the instant pressure method. A root end of 2–4 mm in length was cut off from the root and washed in distilled water. The piece was placed in a drop of 45% acetic acid and crushed with a glass spatula under a coverslip. The cells were observed in interphase, prophase, metaphase, anaphase, and telophase in an Axioskop 40 (Zeiss) light microscope under 40× magnification (Fig. 1).

Cytogenetic indicators. The mitotic index, %, was calculated by the following formula:

$$\text{Mitotic index} = \frac{\text{cells in mitosis}}{\text{total cell count}} \quad (1)$$

The chromosomal aberration analysis revealed disorganization, adhesion, overlap, lagging, colchicine mitosis, and a small percentage of bridging and micronuclei formation (Fig. 2).

For a quantitative description, the index of chromosome aberrations, %, was calculated as follows:

$$\text{Chromosome aberrations} = \frac{\text{chromosome aberrations}}{\text{total cell count}}$$

The cytogenetic studies revealed on average 10 000 cells per variant.

Concentration of malondialdehyde in the onion root cells. The lipid peroxidation in root tissues was determined by the amount of malonic dialdehyde (MDA) interacting with 2-thiobarbituric acid (MDA in fresh mass) [18]. During the experiment, 0.2–0.9 g of onion roots were placed into a polymer 15-cm^3 tube (weighing error ± 0.0001 g). After that, 1 cm^3 of trichloroacetic acid (Merck, Germany) with a mass concentration of 200 g/dm^3 was added to the sample. The mix was stirred and diluted with 3 cm^3 of the same trichloroacetic acid solution. The tubes were centrifuged for 15 min at 1000×g at 4°C. Then, 1 cm^3 of the upper liquid layer was transferred to another tube. After that, 4 cm^3 of a thiobarbituric acid solution (0.5 g of thiobarbituric acid (Diam, Russia)) was poured into 100 cm^3 of trichloroacetic acid solution (200 g/dm^3). The tubes were placed in a 95°C water-bath for 30 min followed by an ice bath. Next, the tubes were placed in a centrifuge for 10 min at 1000×g at 20°C. The resulting solutions were subjected to spectrophotometry in a Cary WinUV 100 spectrophotometer (Varian, USA) at wavelengths of 600 and 532 nm.

Statistical analysis. Statistical processing involved Microsoft Excel 2016 and Statistica 12 software. The root mass indicator was calculated using the nonparametric Mann-Whitney test to compare two means ($P \leq 0.05$). Fisher's test ($P \leq 0.05$) quantified the differences in data with a binomial distribution, i.e. mitotic index and frequency of chromosome aberrations.

RESULTS AND DISCUSSION

The research tested the antioxidant effect of water-diluted apple juice on *Allium cepa* roots after sorbic acid-induced oxidative stress. Antioxidants of plant origin could delay or prevent lipid oxidation because they inhibited the development and accumulation of free radicals [19]. However, sorbic acid is known to trigger the dose-dependent development of oxidative stress and increase the malonic dialdehyde (MDA) content in root tissues [20]. Concentrated solutions of apple juice activated lipid oxidation during the *A. cepa* test [7]. Therefore, the initial task was to select the optimal concentrations of sorbic acid and juice to obtain the maximal antioxidant effect. The onion samples spent 48 h incubating in solutions of sorbic acid and apple juice: 100 mg/L of sorbic acid was diluted with brand A apple juice as 1:2, 1:5, and 1:9. After the incubation, the

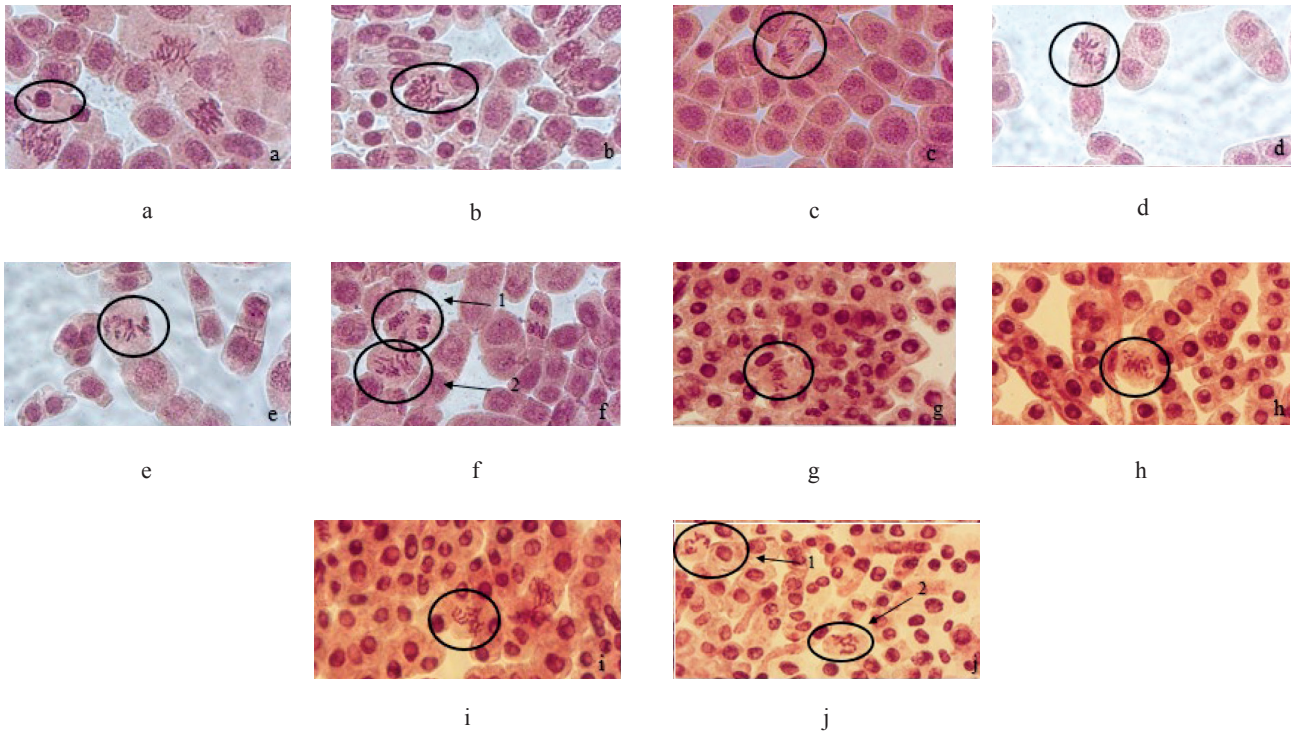
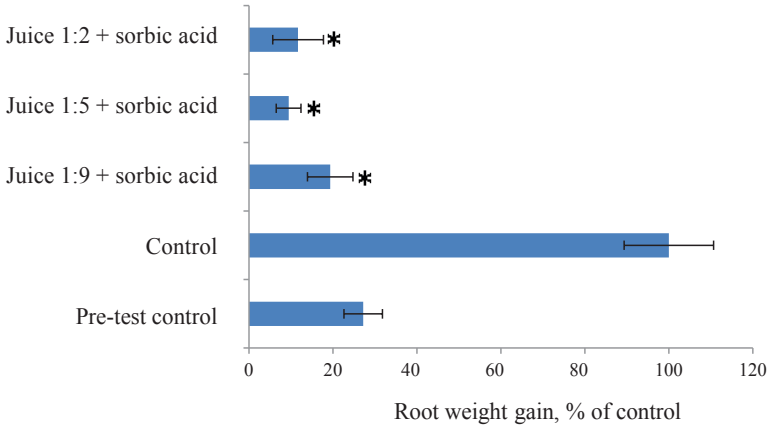


Figure 2 Chromosome aberrations: a) lagging in telophase; b) metafhase with chromosome loss; c) lagging in anaphase; d), e), and i) disorganization in metaphase; f) multipolar mitosis and disorganization in metaphase; g) disorganization in metaphase; h) metafhase with chromosome loss; j) mitosis или colchicine mitosis



* statistically significant difference from control ($P < 0.05$); error bars determine the value of the standard deviation

Figure 3 Decrease in weight gain of onion roots after treatment with brand A juice and sorbic acid (100 mg/L)

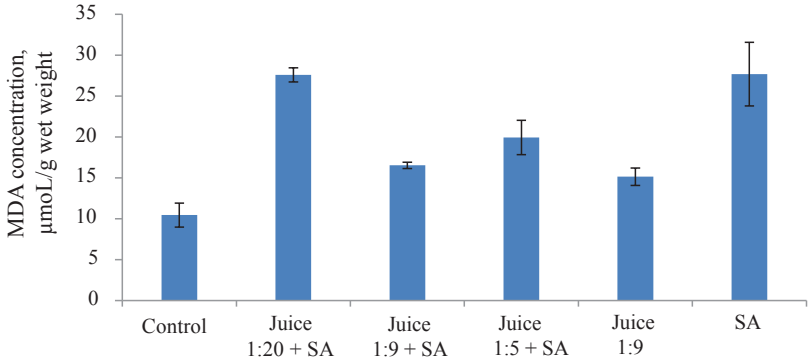


Figure 4 MDA in the roots treated brand A apple juice and sorbic acid (SA, 50 mg/L)

mass of the roots remained the same. In fact, they turned yellow and mucous, which meant that the doses had an acute toxic effect (Fig. 3).

In the next experiment, the treatment time and the acid concentration were halved, and the juice samples were diluted as 1:20, 1:9, and 1:5. Figure 4 shows that the 1:9 juice solution provided the maximal protective effect under oxidative stress caused by a 50 mg/mL solution of sorbic acid. In these samples, the level of MDA was lower by 43% than in the samples with the same concentration of sorbic acid.

The obtained data confirmed the results described in [21], where apple juice in rats' diet decreased the level of MDA in their blood plasma. The phenolic compounds and dietary fiber of apple juice proved to reduce the lipid oxidation in humans as well [1, 22, 23].

The dose-dependent decrease in MDA was revealed only in the first two, more diluted juice solutions (Fig. 4). In 1:5 juice samples, this indicator increased again. This effect was associated with carbohydrates, which are known to have prooxidant properties at this concentration during the *A. cepa* test [7]. Therefore, these data also confirmed that the maximal antioxidant activity of apple juice depended not only on its biologically active compounds, but also on the concentration of carbohydrates.

Some recent research featured the effect of fructose on the redox balance in the organs of the central nervous system. Rat studies revealed an increase in lipid oxidation of brain tissues after both short-term and long-term intake of this carbohydrate [24]. These animal models showed the same results as the abovementioned plant bioassays for the prooxidant properties of apple juice carbohydrates. Therefore, the *A. cepa* test proved to be a reliable research method for the molecular mechanisms of antioxidant and prooxidant properties of apple juice.

Growth indicators demonstrated no significant differences after the onions were treated with solutions of juice and sorbic acid (Table 1). However, previous research revealed that the increase in juice concentration had an adverse effect on onion root cell proliferation [7]. However, the decrease in the mitotic index against the increase in the juice proportion was not dose-dependent (Table 1). Both juice concentrations, 1:20 and 1:9, had the same values of this indicator. Probably, the maximal antioxidant status of the samples diluted 1:9 had protected the proliferative processes by reducing the effects of oxidative stress.

Similar conclusions were reported in a publication about the effect of antioxidants on bisphenol-induced oxidative stress in mouse spermatozoa [25]. Antioxidants preserved the motility of these germ cells, improved the fertilization process, and prevented premature development of the resulting fetus.

The low values of the mitotic index meant a low proportion of dividing cells in the experimental samples with mixes of juice and sorbic acid (Table 1). Therefore, no comparative analysis of chromosomal aberrations was necessary.

The next stage featured the antioxidant potential of various juice brands diluted 1:9 after 24 h of sorbic acid-induced oxidative stress. Juice brands A, B, and C in the mix reduced the level of MDA by 23, 26, and 26%, respectively (Fig. 2). Juice brands B and C also revealed some antioxidant activity; however, the differences between the experimental samples in MDA values were insignificant (3%). In the experimental mixes, the root masses were very similar and minimal, while the values of the mitotic index showed some statistically significant differences (Table 2).

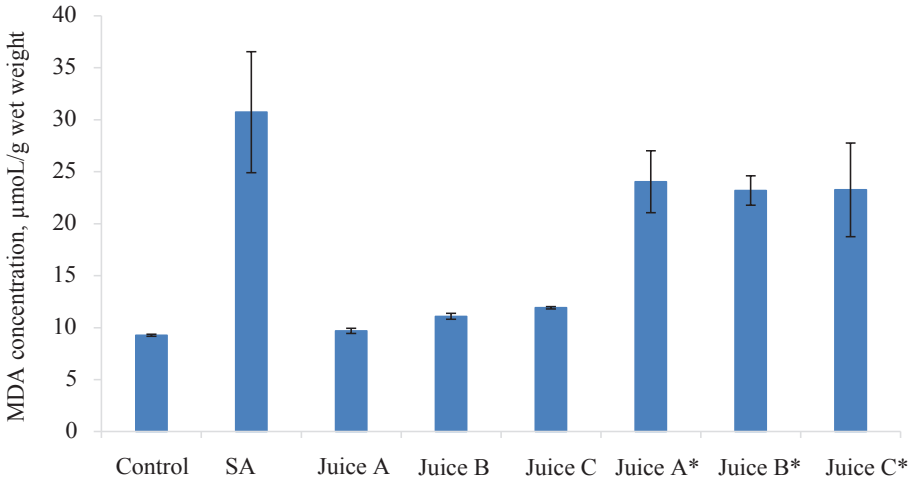
Phenolic compounds are mainly to be found in apple peel and pulp cell walls [1, 26]. Therefore, the processed and clarified juices had some residual differences in antioxidant activity in relation to lipid oxidation. Nevertheless, the bioassay was able to register a rather high antioxidant activity even in these non-pulp juices. The similar MDA values could also be explained by the absence of the pulp as the main source of phenolic compounds.

The results indicated an acute toxic effect (Fig. 3) and an increase in the level of lipid oxidation (Fig. 5) in the mixes of various juices and sorbic acid at concentrations of 100 and 50 mg/L, respectively. For some juice-containing drinks, domestic regulatory documents state much greater permissible concentrations of this preservative, ≥ 1 g/kg. Therefore, sorbic acid can reduce the initial antioxidant potential of these products, but not the content of phytochemical compounds. These data are important if the production

Table 1 Root weight gain, mitotic activity, and frequency of chromosome aberrations in onion root meristem cells after incubation in solutions of brand A juice, sorbic acid (SA), and their mixes

Experiment	Root weight gain, g/onion, mean ± SE*	Mitotic index, %, mean ± SE	Chromosome aberrations per total cells, %, mean ± SE
Control	0.296 ± 0.048^{a}**	8.70 ± 0.24^{a}	0.26 ± 0.04^{a}
Juice 1:20 + SA, 50 mg/L	0.189 ± 0.034^{ab}	1.08 ± 0.11^{b}	0.02 ± 0.02^{b}
Juice 1:9 + SA, 50 mg/L	0.162 ± 0.029^{bc}	1.01 ± 0.08^{b}	0.08 ± 0.02^{c}
Juice 1:5 + SA, 50 mg/L	0.138 ± 0.032^{bcd}	0.40 ± 0.06^{c}	0.04 ± 0.02^{bc}
Juice 1:9	0.109 ± 0.012^{bcd}	1.40 ± 0.09^{d}	0.21 ± 0.03^{ad}
SA, 50 mg/L	0.243 ± 0.021^{ab}	5.86 ± 0.21^{e}	0.18 ± 0.04^{d}

*SE – standard error, ** – values marked by the same letter have no significant statistic difference ($P < 0.05$)



Note: Vertical error bars indicate the value of the standard deviation; * marks the incubation experiments with mixes of juices and sorbic acid (SA, 50 mg/L)

Figure 5 MDA in roots treated with various apple juice brands

Table 2 Root weight gain, mitotic activity, and frequency of chromosome aberrations in onion root meristem cells after incubation in solutions of juices and their mixes with sorbic acid (SA)

Experiment	Root weight gain, g/onion, mean ± SE*	Mitotic index, %, mean ± SE	Chromosome aberrations per total cells, %, mean ± SE
Control	0.236 ± 0.030^{a}**	8.75 ± 0.24^{a}	0.29 ± 0.05^{a}
SA, 50 mg/L	0.211 ± 0.034^{ab}	5.17 ± 0.19^{b}	0.18 ± 0.04^{b}
Juice A	0.139 ± 0.016^{bc}	2.55 ± 0.13^{c}	0.05 ± 0.02^{c}
Juice B	0.146 ± 0.016^{bcd}	1.10 ± 0.09^{d}	0.11 ± 0.03^{b}
Juice C	0.184 ± 0.024^{abcde}	0.99 ± 0.08^{d}	0.01 ± 0.01^{d}
Juice A + SA, 50 mg/L	0.155 ± 0.021^{bcdef}	0.48 ± 0.05^{e}	0.020 ± 0.001^{cd}
Juice B + SA, 50 mg/L	0.143 ± 0.030^{bcdefg}	0.58 ± 0.07^{ef}	0.02 ± 0.01^{e}
Juice C + SA, 50 mg/L	0.171 ± 0.012^{abcdefg}	0.56 ± 0.07^{ef}	0.04 ± 0.02^{e}

* SE – standard error, ** – values marked by the same letter have no significant statistic difference ($P < 0.05$)

Table 3 Root weight gain, mitotic activity, and frequency of chromosome aberrations in onion root meristem cells before and after recovery treatment in juice solutions

Experiment	Root weight gain, g/onion, mean ± SE*	Mitotic index, %, mean ± SE	Chromosome aberrations per total cells, %, mean ± SE
		Before recovery treatment	
Control	0.799 ± 0.089^{a}**	8.52 ± 0.27^{a}	0.02 ± 0.01^{a}
Juice A	0.561 ± 0.056^{ab}	3.06 ± 0.19^{b}	0.12 ± 0.04^{b}
Juice C	0.540 ± 0.048^{bc}	3.64 ± 0.18^{c}	0.08 ± 0.03^{bc}
Juice D	0.597 ± 0.060^{abc}	3.95 ± 0.20^{c}	0.15 ± 0.04^{abc}
		After recovery treatment	
Control	1.060 ± 0.082^{a}	7.82 ± 0.28^{a}	0.16 ± 0.04^{a}
Juice A	0.791 ± 0.088^{ab}	7.99 ± 0.25^{ab}	0.43 ± 0.06^{b}
Juice C	0.827 ± 0.094^{abc}	8.08 ± 0.27^{ab}	0.99 ± 0.10^{c}
Juice D	0.944 ± 0.095^{abc}	6.93 ± 0.25^{c}	0.41 ± 0.06^{b}

technology provides for this preservative. However, only bioassay can determine how these effects interact.

Our experiments on the toxic potential of different juice brands were aimed at a comparative assessment of their side effects on the growth and the cytological, cytogenetic, and biochemical parameters of onion roots. We found no scientific publications that featured the *A. cepa* test as a means of researching the toxic effect of apple juice. The main task was to obtain data on possible irreversible violations of these processes. In case of complete or partial irreversibility after the juice treatment, the detoxification systems of the plant organism failed to cope with the load, and these negative phenomena might progress in the future.

The previous experiments had a high toxic load because of sorbic acid (Table 2). In this experiment,

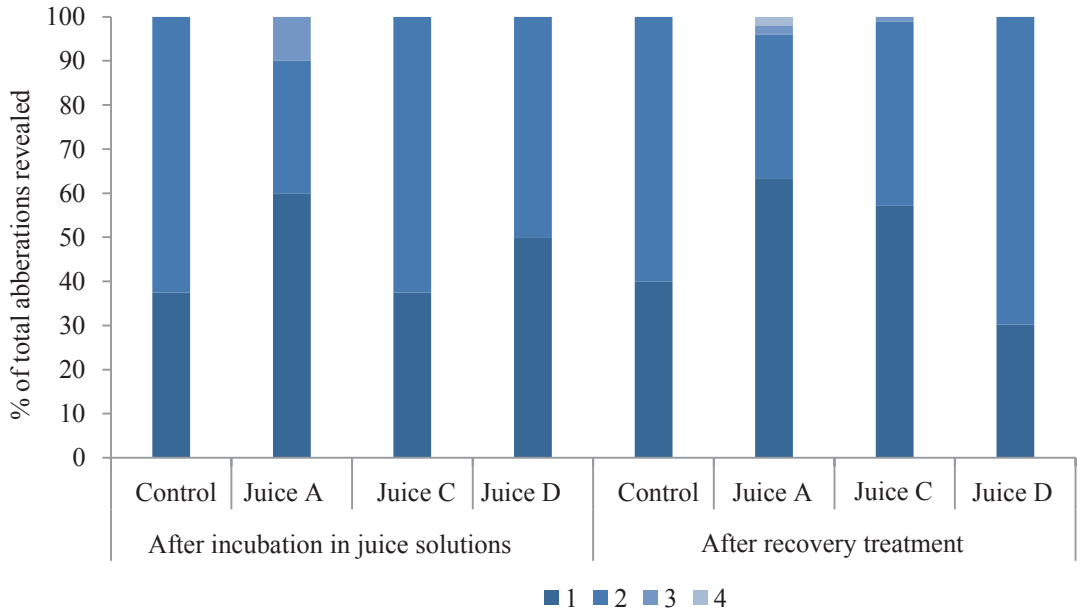
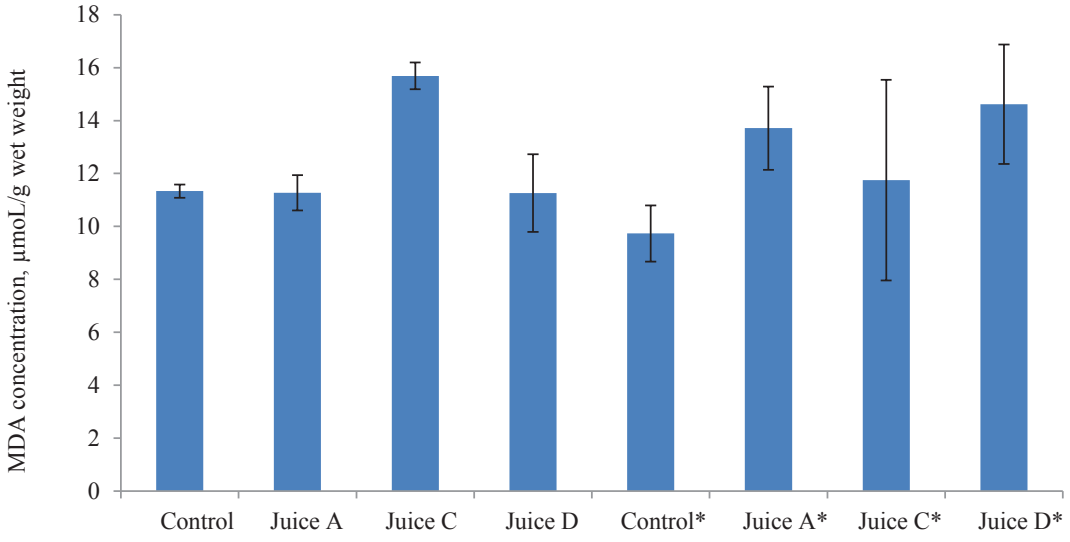


Figure 6 Chromosomal aberrations in meristem cells of onion roots before and after recovery treatment in juice solutions: 1) disorders of chromosome segregation (overlap, lag); 2) anomalies of mitotic apparatus (adhesion, multipolar mitosis); 3) aberrations of clastogenic character (bridges, fragments); 4) miscellaneous (fragmentation, agglutination, pulverization)



Note: Vertical error bars indicate the value of the standard deviation, * – marks incubation in acid solutions followed by germination in bottled water

Figure 7 MDA in roots treated with aqueous solutions of juices

the treatment time with juice solutions reached 48 h. After recovery treatment, the average weight of the roots was by 11–25% lower than that in the control samples, but this difference was not significant (Table 3). Mitotic index had the same trend, except for the brand D juice samples, although this indicator differed from the control by only 11%. However, the cytogenetic analysis showed a significant increase in chromosomal aberrations in all the experimental groups, while the maximal growth by more than six times was recorded in the brand C juice samples.

Figure 6 shows the chromosome aberrations found in the apical meristem of the onion roots after incubation in juice solutions, as well as after incubation and subsequent regeneration in bottled water. No statistically significant differences ($P \leq 0.05$) in chromosomal disorders were revealed before or after recovery treatment in bottled water. However, juice A samples demonstrated all kinds of aberrations after recovery treatment

Thus, all the experimental samples revealed irreversible significant genotoxic effects (Table 3), represented mostly by chromosome disorganization

in metaphase, lagging in anaphase, metafhase with chromosome loss, and lagging in telophase (Fig. 6). Disorganization of chromosomes in metaphase, for instance, was a typical irreversible side effect of benzoic acid on onion roots [20].

After recovery treatment, MDA content was higher in all the experimental variants by 21–51% compared with the control values (Fig. 7). This indicator also demonstrated the irreversible nature of the identified adverse effects after exposure to juice solutions.

Thus, the maximal negative effects after recovery treatment were recorded when analyzing the values of the mitotic index and MDA in the D juice samples and the level of chromosome aberrations in the C juice samples. If the first two indicators differed from the control only by tens of percent, the latter differed by several times in all the experimental variants. In the juice C samples, the level of cytogenetic disorders was two times higher compared to samples A and D. This biomarker requires more attention when assessing the genotoxic potential of this product, both phytochemical and technological.

CONCLUSION

The research featured a new bioassay method for determining the antioxidant potential of processed apple juice. The juice reduced the lipid oxidation in onion roots to 40% after oxidative stress induced by sorbic acid. The antioxidant potential in juice solutions depended on the ratio of biologically active compounds and carbohydrates.

The research included a comparative analysis of three juice brands. Sorbic acid had a possible negative effect on the quality of juice-containing products: even 50 mg/L reduced the antioxidant profile of the finished product. When the concentration of sorbic acid reached 100 mg/L, its effect became toxic, and onion roots died. No side toxic subchronic effects on the weight gain were registered after onion roots were treated with three juice brands. However, one of the three juices demonstrated an irreversible decrease in the proliferative index by 11%.

The cytogenetic analysis of the root meristem revealed the maximal adverse side effect: chromosomal aberrations increased in all experimental groups. For one brand, these disorders increased by more than six times. In general, the *Allium cepa* bioassay of toxic subchronic effects provided reliable results for side effects in apple juice production.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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