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Research article

IDENTIFICATION OF GENETICALLY MODIFIED ORGANISMS IN FOODS OF PLANT ORIGIN AS A WAY TO CONTROL HEALTH RISKS FOR CONSUMERS

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Uncontrolled distribution of goods produced by genetically modified plants should be prevented by the state in order to secure food safety in the Russian Federation and to minimize health risks for consumers.

We analyzed foods of plant origin for children to identify components of genetically modified organisms in them. It was done to ensure safety of such foods. The highest specific weight among the analyzed foods belonged to nectars (40.0 %) and juice-containing drinks (36.0 %). Juices and fruit drinks accounted for 12 % each. Genetically modified organisms were determined in foods by identifying regulatory sequences (35S promoter, FMV promoter and NOS terminator) that are widely used in constructions of genetically modified plants. Occurrence of regulatory genetic elements specific for genetically modified organisms was checked in juice products for children by the polymerase chain reaction in real-time mode with hybridization-fluorescent detection of amplification products and with the use of the "AmpliSens GM Plant-1-FL" and "AmpliKvant GM soya-FL" test systems.

The results of this study showed that no analyzed foods of plant origin contained any regulatory sequences (35S, NOS u FMV) indicating presence of genetically modified organisms. Fluorescence through the FAM, Cy5 and ROX channels did not exceed its threshold value. Therefore, we did not detect any violations of the established requirements to occurrence of genetically modified organisms in foods for children. Further investigation that would involve examining a more extensive material is required to ensure proper assessment and control of food contamination with genetically modified organisms in order to ensure food safety.

Keywords: *transgenic plants, genetically modified organisms, promoters, terminators, polymerase chain reaction, food safety, juice foods for children.*

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At present, food safety is a pressing issue given the global population growth [1, 2]. It is an integral component of national security in any state [3]. The Food Safety Doctrine of the Russian Federation was approved by the RF President Order issued on January 21, 2020 No. 20. The item 7z in it stipulates the necessity to prevent any imports of genetically modified organisms into the country as a key national interest in providing food safety. It is prohibited to sow, grow, cultivate or distribute such products in Russia; the Order also highlights the necessity to control imports and distribution of foods produced with using genetically modified organisms[1].

Genetically modified cultures are being used more and more often to increase crop capacity of plants and make them more resistant to harmful factors [4–6]. There are varieties of fruit and vegetables that have been created in laboratories by up-to-date genetic engineering technologies [7–9]. Nowadays genetically modified organisms can be found in most foods, juice products included.

The juice products market is developing dynamically and it can provide consumers with a wide and diverse range of juices, nectars, juice-containing drinks and fruit drinks[2] [10–12]. Juice products are considered a source of necessary nutrients and biologically active compounds [13–16]. Manufacturers issue multiple innovative products on the market all over the world. They are currently switching to more healthy ingredients in the process given a growing consumer interest in healthy diets. Technological processes and receipts are being developed and juice products are being enriched with vegetable components and vitamins [17].

Since 2016, the legislation in Russia strictly forbids any commercial growing of genetically modified cultures; still, their imports are allowed provided they have been registered in full conformity with the state registration procedure[3]. However, it is forbidden to use genetically modified organisms in foods for children, juices included. At present, the mandatory requirements aimed at providing juice products safety are stipulated in the Technical Regulations of the Customs Union CU TR 023/2011 “The Technical Regulations for juice products made of fruit and vegetables”[4]. Most juices and juice-containing drinks that are sold in the country are manufactured in Russia from imported concentrated purees and juices. Imported raw materials account for 80 % in juice products manufactured in the country [18] and this share has remained stable over the last 20 years. Many fruit cultures that are used in juice products manufacturing cannot be grown in Russia due to unsuitable climatic

[1] Ob utverzhdenii Doktriny prodovol'stvennoi bezopasnosti Rossiiskoi Federatsii: Ukaz Prezidenta RF № 20 ot 21.01.2020 g. [On Approval of the Food Safety Doctrine of the Russian Federation: The RF President Order No. 20 issued on January 21, 2020]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/564161398?section=text (June 07, 2022) (in Russian).

[2] Rynok sokov i nektarov v Rossii. Tekushchaya situatsiya i prognoz 2022–2026 gg. [Juice and nectar market in Russia. The current situation and forecasts for 2022–2026]. *Alto Consulting Group (ACG)*. Available at: https://alto-group.ru/otchot/rossija/285-rynok-sokov-i-nektarov-v-rossii-tekuschaja-situacija-i-prognoz-2021-2025-gg.html (June 03, 2022) (in Russian).

[3] O vnesenii izmenenii v otdel'nye zakonodatel'nye akty Rossiiskoi Federatsii v chasti sovershenstvovaniya gosudarstvennogo regulirovaniya v oblasti genno-inzhenernoi deyatel'nosti: Federal'nyi zakon № 358-FZ ot 03.07.2016 g. [On making alterations into certain legislative acts of the Russian Federation regarding development of state regulation in the sphere of genetic engineering: The Federal Law No. 358-FZ issued on July 03, 2016]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/420363719?section=text (June 08, 2022) (in Russian).

[4] TR TS 023/2011. Tekhnicheskii reglament na sokovuyu produktsiyu iz fruktov i ovoshchei (utv. Resheniem Komissii Tamozhennogo soyuza ot 9 dekabrya 2011 goda № 882) [CU TR 023/2011. The Technical Regulations for juices made of fruit and vegetables (approved by the Decision of the Customs Union Commission on December 09, 2011 No. 882)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320562?section=text (June 08, 2022) (in Russian).

conditions. Therefore, there is a risk that genetically modified organisms can penetrate the Russian food market without any proper state registration. Besides, there are risks that some products might be falsified, counterfeited or declared inadequately. It is necessary to implement new techniques for determining quality, safety and authenticity of juice products that are more effective than the existing ones.

Our research goal was to identify probable occurrence of genetically modified organisms in foods of plant origin for children in order to ensure food safety.

Materials and methods. Genetically modified organisms were identified in foods by the polymerase chain reaction in real-time mode with hybridization-fluorescence detection. Overall, we analyzed 50 samples of foods of plant origin for children. The highest specific weight among the analyzed foods belonged to nectars (40.0 %) and juice-containing drinks (36.0 %). Juices and fruit drinks accounted for 12 % each.

The first stage involved DNA extraction in accordance with the recommendations provided by the manufacturer of the "MagnoPrime FITO" reagent kit ("NekstBio" LLC. Moscow). At the next stage, the obtained samples were amplified with CFX96 (Bio-Rad, USA) as per the following program: 1 cycle: 95 °C for 900 sec; 2 cycles: 95 °C for 15 sec, 59 °C for 60 sec. We applied the "AmpliSens GM Plant-1-FL" and "AmpliKvant GM soya-FL" test systems (Rospotrebnadzor's Central Scientific Research Institute for Epidemiology) in the process for qualitative and quantitative determination of genetically modified ingredients of plant origin in foods accordingly. Amplification completed, we then detected intensity of fluorescence as per channels that corresponded to the FAM, HEX, ROX, Cy5 dyes. The results were estimated as per an intersection between the fluorescence curves with the threshold line preset at a certain level. The result was considered valid in case correct results were obtained for positive and negative controls of DNA extraction and amplification in accordance with the instructions provided with the reagent kit. Fluorescence curves were analyzed with CFX Manager software package.

Results and discussion. A wide variety of fruits, vegetables and berries are used as raw materials in juice manufacturing. Most analyzed juice products were samples made from apple, peach, tomato or orange concentrated juice and (or) purees as well as from multi-fruit concentrated products. Rather few samples contained grapes, cherry, black current, bananas, apricots, peaches, pears, grapefruits, cowberries, blackberries, litchi, and granadilla.

Our study focused on identifying DNA promoters 35S, FMV and NOS terminator in juice products for children. Their occurrence would indicate that genetically modified organisms were present. The results showed that the analyzed samples did not contain any specific sequences and that fluorescence as per FAM, Cy5 and ROX channels did not exceed the threshold level. Still, as we performed the polymerase chain reaction in real-time mode, we detected accumulating fluorescence as per the channel for controlling plant DNA (HEX) occurrence in 86.0 % of the analyzed samples. Nectars accounted for 39.5 % of such samples; juice-containing drinks, 37.2 %; juices, 14.0 %; and fruit drinks, 9.3 %. The Figure shows levels of fluorescence at the last amplification cycle for five food samples as per all the analyzed channels. Obviously, these levels exceed the threshold value only as per HEX channel indicating that plant DNA occurs in the analyzed samples.

Our examination with using "AmpliKvant GM soya-FL" did not establish any growth in fluorescence signals as per FAM and HEX channels. This confirms absence of any regulatory sequences typical for genetically modified organisms and soya DNA in all the analyzed samples of juice products for children.

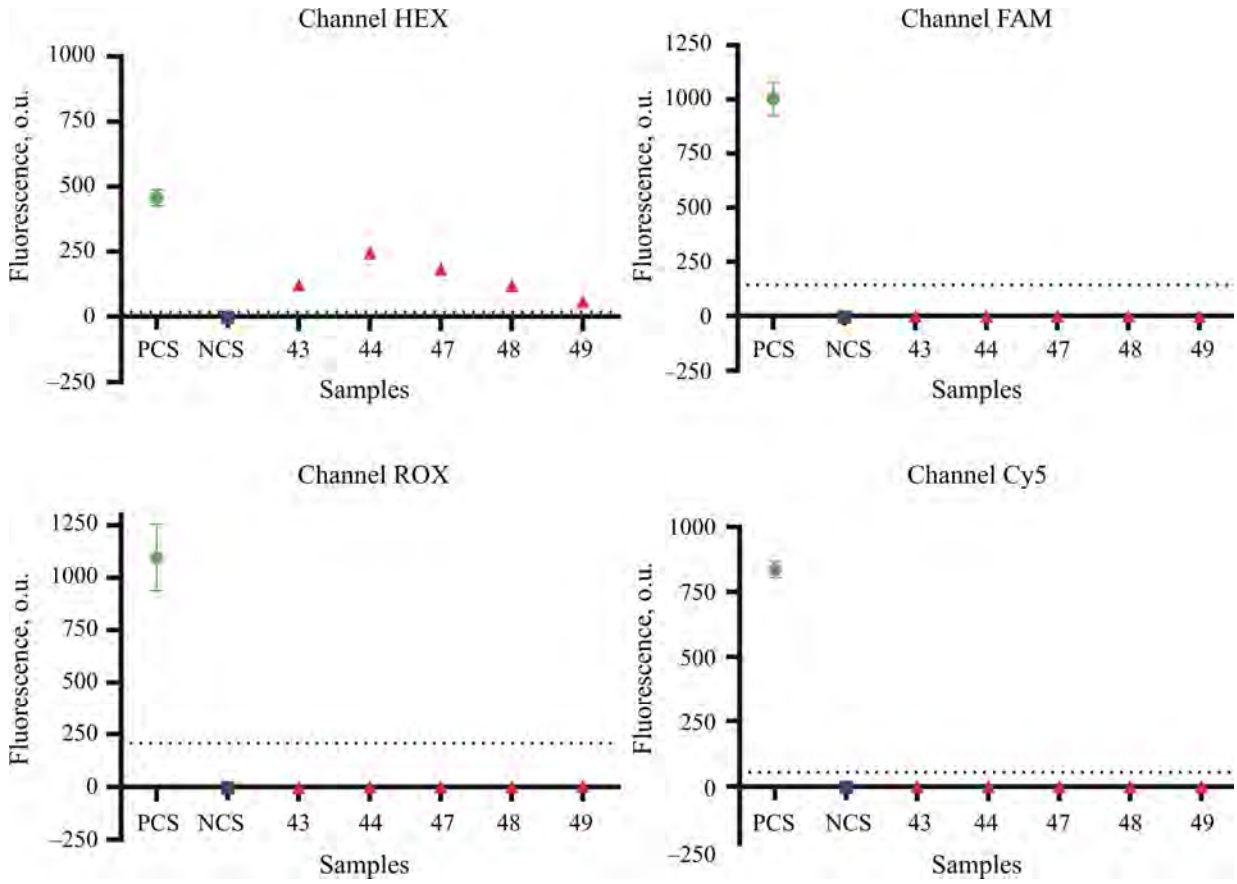

Figure. Levels of fluorescence at the last amplification cycle as per HEX, FAM, ROX, and Cy5 channels: o.u. is optical units; PCS is positive control sample; NCS is negative control sample

An issue related to probable risks of using genetically modified organisms is still being discussed [19, 20]. Food safety should include concerns for health of elderly people, pregnant women and nursing mothers as well as children. In the Russian Federation, there is the valid moratorium on use of genetically modified organisms in foods for children[5]. In this study, we did not detect genetically modified organisms in any sample of juice products for children. This means that all the analyzed products conform to the requirements stipulated in the existing RF legislation.

Similar results were obtained in an earlier study accomplished in Astrakhan that did not detect any NOS terminator sequences in foods for children, juices included [21]. Most juices do not contain any genetically modified organisms since transgenic fruit and vegetables are grown in much smaller quantities than basic agricultural crops. However, recently there has been an ascending trend in use of biotechnologies in fruit and vegetable production [22, 23]. Thus, a new apple variety grown with using biotechnologies is already available on the US market. This pro-

[5] TR TS 021/2011. O bezopasnosti pishchevoi produktsii (s izmeneniyami na 14 iyulya 2021 goda) (utv. Resheniem Komissii Tamozhennogo soyuza ot 9 dekabrya 2011 goda № 880) [CU TR 021/2011. On food products safety (last amended on July 14, 2021) (approved by the Decision of the Customs Union Commission on December 09, 2011 No. 880)]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320560?section=text (June 09, 2022) (in Russian).

duct's name is "Arctic apple"; it was genetically modified to prevent browning after slicing thus making a fruit look fresh. In October 2020, a genetically modified pineapple was introduced on the market. The fruit is sweeter and has bright pink pulp [23]. Transgenic papaya was first produced as far back as in 1990ties; a GMO-containing variety of this fruit is more resistant to the papaya ringspot virus [24]. While citrus production in the USA is trying to overcome the incurable bacterial disease known as Huanglongbing ("citrus greening disease") that destroys orange trees in the country, experts in the field are searching for a similar solution to the issue [25, 26].

Conclusions. We analyzed juice products for children in this study. As a result, we did not detect any regulatory genetic elements typical for genetically modified organisms. Therefore, the existing legislative requirements regarding occurrence of genetically modified components in foods for children have not been violated. Further investigations on wider samplings are required for proper assessment and control of food contamination with genetically modified organisms in order to ensure food safety.

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Competing interests. The authors declare no competing interests.

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