

GMO ON THE BULGARIAN MARKET - 2023

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Summary

The rise of biotechnology has brought genetically modified organisms (GMOs) into farming and food production, sparking significant public discussion. With more GMOs entering the market, the workload for testing labs has surged. This study thoroughly examines food products available in Bulgaria to determine the extent of GMO presence. Its goal is to offer an unbiased view of GMO distribution and variety in the Bulgarian market as of 2023. The aim of this study is to conduct an impartial assessment of genetically modified organism presence in the Bulgarian market, alongside verifying if products adhere to GMO labeling regulations. This research focused on examining 79 distinct samples of food products available in the Bulgarian market. Molecular genetic techniques utilizing quantitative polymerase chain reaction (qPCR) were employed for analysis. In 91% of the samples no GMO was detected, 9% were positive for various GMOs authorized in the EU, but all of them were below the LOQ or content level below the threshold of 0.9% - GMO content for mandatory labeling. The findings from the analysis of samples for genetically modified organisms offer crucial insights into the present market situation and the level of adherence to regulatory standards. While the overall percentage of positive samples and contamination levels remain low, falling below the mandatory labeling threshold of 0.9% GMO-content, there is a noticeable increase in the variety of detected GM events.

Key words: *GMO, Bulgaria, 2023*

Introduction

There is a noticeable lack of comprehensive research focusing on the impact and regulation of GMOs in the EU market, with very few studies addressing this issue in depth, and those that exist are not recent. Similarly, while the European Union has some overarching GMO policies, research exploring their direct effects on local markets, especially in Bulgaria, remains limited and outdated. This gap underscores the need for more up-to-date, region-specific studies to better understand the current dynamics of GMO adoption and consumer perception within Bulgaria and the broader EU context.

As the number of genetically modified organisms (GMOs) entering the market rises, testing laboratories face increased demand (Querci et al. 2010, Scholtens et al. 2013). Ready-to-use PCR-based detection systems, such as Pre-spotted Plates (PSP), offer a solution by reducing assay time and increasing throughput. The Screening PSP, designed to detect all EU-authorized GMOs in a single PCR experiment, employs 16 validated assays. During the screening phase, samples are examined for the presence of GM material, using assays targeting widely-used regulatory sequences, genes, or constructs. This step is crucial for cost and time optimization, particularly when investigating the potential presence of numerous GMOs (Angers-Loustau et al. 2014, Broothaerts et al. 2023). The optimal GMO screening strategy should aim to detect all known and pending events, minimize the need for additional testing, and potentially uncover unknown or unauthorized GM events.

PSPs consist of 96-well plates preloaded with primers and probes for selected assays, enabling multi-target assays with minimal preparation. Developed by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) of the Joint Research Center (JRC) in 2009, PSPs have since been shown to be effective in various GMO testing scenarios (Kluga et al. 2012). The plates used in this analysis cover 7 taxa, 5 elements, 1 construct, and 3 event-specific analyses, selected from the GMOMETHODS database (<http://gmo-crl.jrc.ec.europa.eu/gmomethods/>) to maximize coverage of authorized GMOs with minimal analyses.

The aim of this study is to conduct an unbiased examination of genetically modified organism presence in the Bulgarian market, alongside verifying if products comply with GMO labeling regulations.

Materials and methods

A total of 79 samples were analyzed such as soy, soy granulate, popcorn corn, waffles, various meat products, etc. The selection of products for analysis was carried out based on various criteria, including food categories, brands and retail chains.

The study paid attention to the diversity of food products consumed by consumers in Bulgaria, taking into account both basic products and those with a more specific purpose. This selection plays a key role in obtaining representative data on the presence of GMOs in different market segments.

Molecular genetic methods:

- DNA Extraction from food products:

DNA extraction was performed using a Macherey-Nagel™ NucleoSpin™ Food Column (Thermo Fisher Scientific, Product code. 11972402) from food samples, according to the manufacturer's recommendations.

- Quantitative and qualitative evaluation of extracted DNA

The quality and quantity of the extracted nucleic acid was assessed by spectrophotometric analysis. DNA concentration was measured spectrophotometrically (Agilent 8453 UV-Visible Spectroscopy System, Agilent Technologies) at wavelengths of 260 nm and 280 nm.

- Qualitative determination of DNA by real-time PCR

Qualitative determination of DNA by real-time PCR was performed according to the standard БДС EN ISO 21569:2005/A1:2013 Food products. Methods of analysis for the detection of genetically modified organisms and their products. Nucleic acid-based qualitative methods (ISO 21569:2005/Amd 1:2013).

In order to reduce the analysis time and increase the capacity for qualitative determination of DNA by real-time PCR, ready-to-use multi-purpose detection systems based on PCR - (PSP) are used.

- DNA quantification by real-time PCR

DNA quantification by real-time PCR was performed according to the БДС EN ISO 21570:2005/A1:2013 Food standard. Analytical methods for the detection of genetically

modified organisms and genetically modified products. Quantitative methods using nucleic acid (ISO 21570:2005/Amd. 1:2013).

Standard samples with known amounts of GMO were used to create a calibration curve. This allows the results of the qPCR cycles to be converted into quantitative information for the presence of GMOs in the sample.

Statistical methods of analysis:

- The primary processing of the real-time polymerase chain reaction data was done with the software product of Applied Biosystems - SDSv2.2.2.
- The results were processed with Microsoft®Office Excel 2010 (Microsoft Office Professional Edition 2010). The created spreadsheets are checked for correct entry of information.

Results

79 samples were analyzed, such as corn flour, popcorn, soy protein isolate, soy granulate, sweet corn, snacks, waffles, sausages, etc. In 91% of the samples no GMO was detected, 9% were positive for various GMOs authorized in the EU (Fig. 1), but all of them were below the LOQ or content level below the threshold of 0.9% GMO content for mandatory labeling.

Fig. 1 Distribution of GMOs in the examined samples for 2023

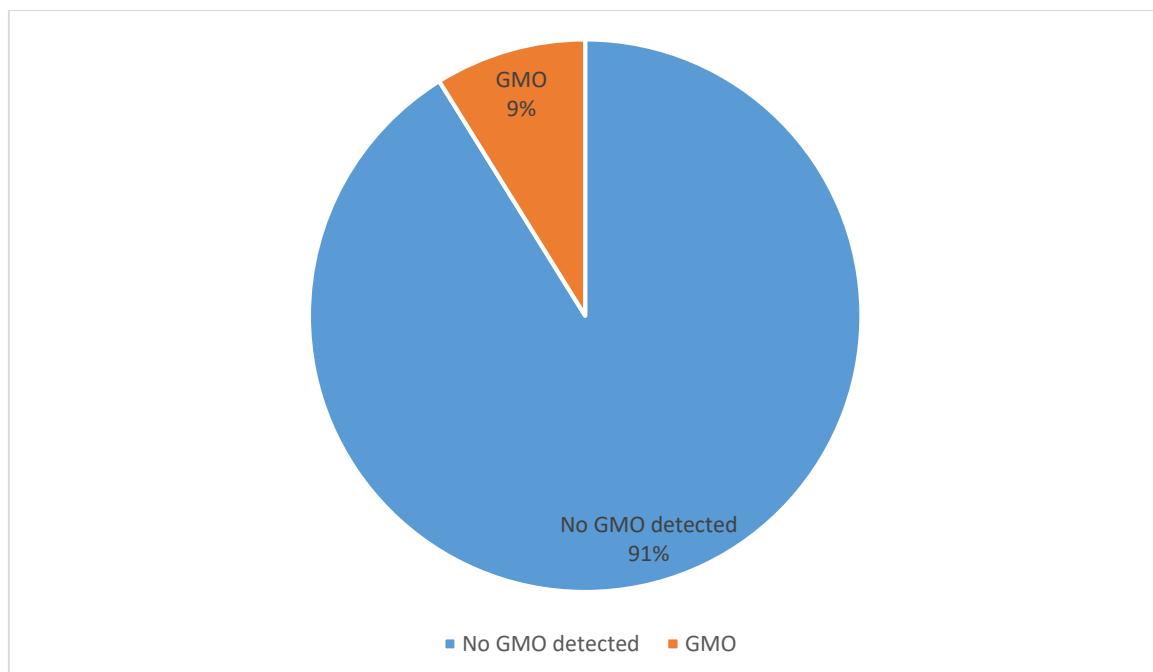
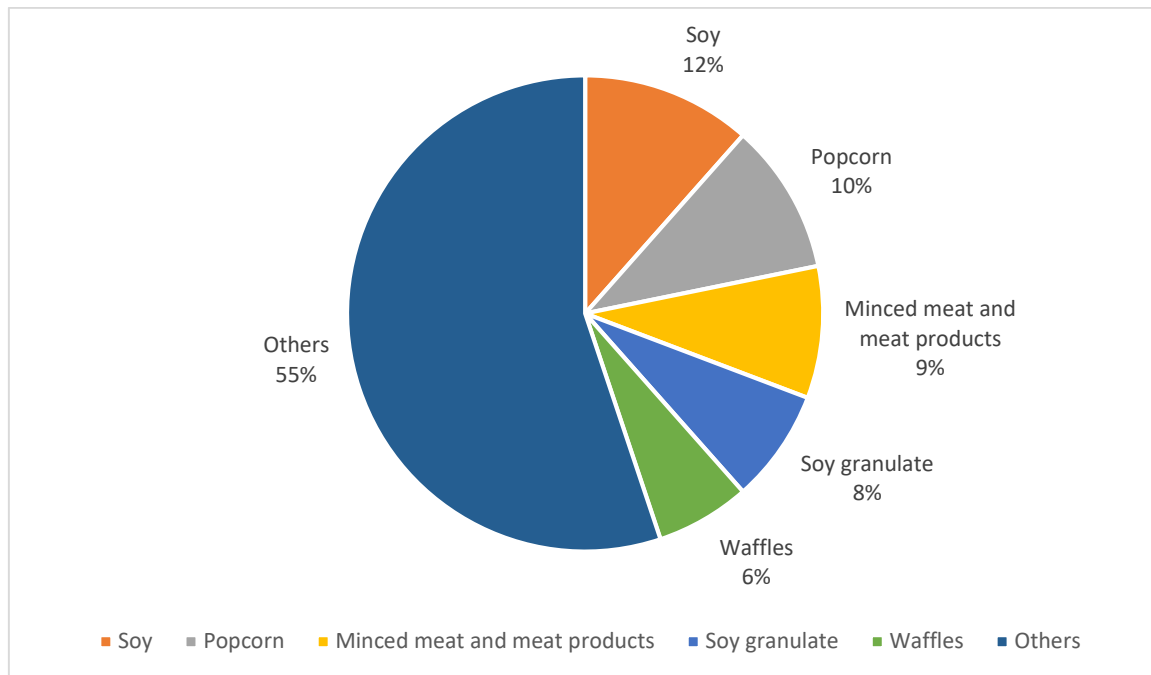


Fig. 2 presents the most analyzed types of samples. Among them, 12% are soy, 10% - popcorn, 9% - minced meat and its products, 8% - soy granulate and 6% - waffles. Soybeans, corn and some ingredients in meat products are frequent targets for genetic modification, so the largest number of these types of products were analyzed.

Fig. 2 Most Analyzed Sample Types 2023



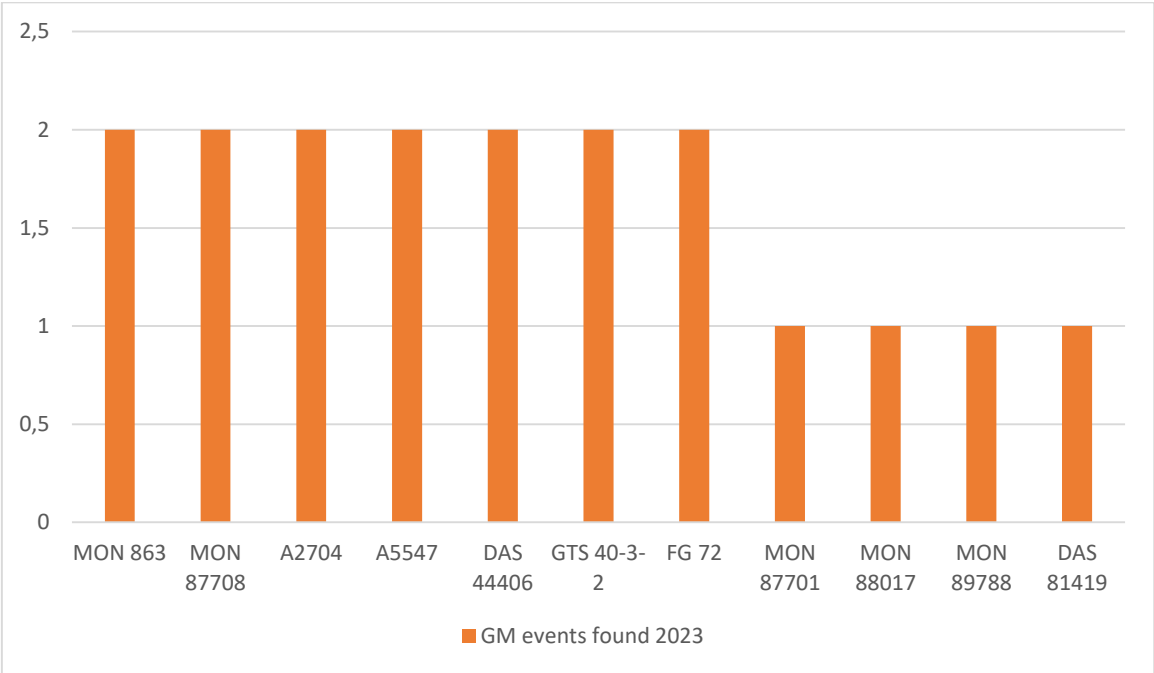
A large number of different GM events were identified in the positive samples (Fig. 3), with more than one GM event detected in three of the samples, highlighting the complexity of genetically modified content in some products.

In agriculture, it's not uncommon for crops to be engineered with multiple traits for improved characteristics (e.g., insect resistance, drought tolerance, herbicide tolerance). The detection of multiple GM events in a product means that the genetic modifications can interact in ways that might be difficult to anticipate, and this complexity may require careful monitoring and regulation.

In the EU, stringent regulatory frameworks ensure that only certain GM crops are authorized for use, often leading to lower levels of GM content in products. In contrast, in countries like the U.S., where GMOs are more widely accepted, multiple GM events can be present in food products without mandatory labeling unless specific thresholds are exceeded. This

discrepancy in GM crop regulation reflects broader global differences in the adoption and acceptance of biotechnology in agriculture, with the U.S. and other countries exhibiting greater diversity in GM events due to looser regulations.

Fig. 3 GM events found 2023



The products most commonly found to be GMO are popcorn, soy protein, and soy granulate. The prevalence of these products as GMO is largely due to agricultural and industry trends focused on crop efficiency, economic value, and consumer demand.

Among the products where GMOs were most frequently found, popcorn stood out, with the most commonly detected GM event being MON 863. This particular GM event involves a genetically modified variety of corn, specifically engineered to improve the crop’s resistance to pests, enhance yield, and increase overall plant resilience against diseases.

MON 863 is primarily used in the production of animal feed and food supplements, where its genetic modifications provide practical benefits, such as reducing the need for chemical pesticides and increasing the efficiency of crop production. This strain of corn has been widely adopted in regions with large-scale corn farming, particularly in the U.S., where GM crops dominate the market. The detection of MON 863 in various samples highlights the prevalence of genetically modified corn in food and feed products, even in seemingly simple

items like popcorn.

All positive samples were below the LOQ or content level below the threshold of 0.9% GMO content for mandatory labeling.

The 0.9% value was introduced as a compromise in the legislation and regulations that govern the labeling of genetically modified organisms (GMOs). This percentage represents an agreement between scientific, technical and commercial factors, as well as public concerns and demands for transparency.

The value is not arbitrary and is the result of negotiations and consultations conducted by international and national regulatory bodies. It is designed to strike a balance between providing information to consumers and facilitating producers, while offering a scientific basis for food safety.

Studies conducted in the EU often report results where detected GMO content is below this threshold, meaning that while some products may contain trace amounts of GMOs, they do not meet the 0.9% threshold required for labeling. These findings are consistent across several EU studies that focus on monitoring GMOs in the market, where positive results are typically below the 0.9% limit (Twardowski and Małyska, 2015).

Discussion

Data on food samples analyzed for GMO content in 2023 can be summarized as follows.

91% of the samples do not contain GMOs. 9% of the examined food products tested positive for the presence of GMOs, but all of them were below the LOQ or content level below the threshold of 0.9% GMO content for mandatory labeling.

The potential risks of GM contamination in food products that are below the 0.9% limit - the level established by the European Union for mandatory labeling - are often overlooked, but they still raise several important concerns. While this level is considered to be below the threshold for mandatory labeling, there are various risks and implications associated with even small traces of GM ingredients in food products. The 0.9% threshold ensures that food products with less than this amount of GM content do not need to be labeled as containing GMOs. However, even at these low levels, consumers who are strongly opposed to GMOs may unknowingly consume them. This raises ethical concerns about consumer rights, transparency, and informed choice. For individuals who prefer organic or non-GMO foods for

health, environmental, or ethical reasons, even small amounts of contamination undermine their ability to make fully informed decisions about their food.

The products where GMOs are most commonly found are popcorn, soy protein and soy pellets.

Corn is one of the most widely genetically modified crops cultivated globally, with its genetic makeup altered to enhance certain traits that improve agricultural productivity and pest management. A primary focus of genetic modification in corn has been to engineer resistance to pests, such as by incorporating genes that produce Bt toxin, a naturally occurring insecticide that specifically targets harmful insects like the European corn borer. This modification reduces the need for chemical pesticide applications, making the crop more sustainable and cost-effective for farmers. Additionally, genetically modified corn has been designed for resistance to herbicides, particularly glyphosate, a widely used herbicide. This genetic alteration allows farmers to control weeds more efficiently without damaging the corn itself, thus improving yields and reducing labor costs associated with weed management.

About 94% of soybeans grown in major producer countries like the U.S. are genetically modified (Bøhn et al. 2014). These are commonly modified for herbicide resistance and pest tolerance. Soy protein is derived from soybeans, making it one of the most frequently GMO products in processed food. It is a key ingredient in plant-based foods, protein bars, and supplements. GMO soybeans are cheaper to produce and yield higher output, making them a staple for industrial use. Soy granulate is another processed soy product, often used in meat substitutes and as a protein-rich ingredient in various foods. Like soy protein, soy granulate benefits from the efficiency of GMO soybeans and is favored by manufacturers due to its cost-effectiveness and availability.

Corn and soy are among the top GMO crops globally due to their economic significance and widespread industrial applications. As raw materials for numerous food items, GMO soy and corn derivatives are almost ubiquitous in processed and packaged foods.

Almost half of the products positive for GMOs are not of Bulgarian origin and this is due to one of the main elements of the internal market of the European Union (EU) - the principle of free movement of goods. This fact can be attributed to several factors:

- 1) Bulgaria imports a variety of processed foods from countries where genetically modified organisms (GMOs) are commonly used in agriculture and food

production. Examples include the United States, Brazil, Argentina and some European countries. These products may unintentionally contain trace amounts due to widespread use of GM crops like soybeans, corn, or canola in these countries;

- 2) Bulgarian agricultural policies are restrictive when it comes to GMO cultivation. Bulgaria, like other EU countries, has opted to ban or severely limit the cultivation of genetically modified crops to preserve biodiversity and comply with public sentiment against GMOs. As a result, domestically grown agricultural products are less likely to contain GMOs compared to imported ones.
- 3) Food production and ingredient sourcing are globalized. For example, even if a product is assembled or partially produced in Bulgaria, its ingredients may be sourced internationally. If these ingredients are derived from crops like GMO soy or corn, the final product may test positive for GMOs.
- 4) Countries vary in their regulations and labeling standards for GMOs. Some exporting countries may not require the same level of transparency or control over GM ingredients, leading to GMO content in products that are then exported to Bulgaria.
- 5) GM crops are often less expensive to produce due to higher yields and pest resistance. Companies looking to reduce costs might opt to use GMO ingredients in products destined for the Bulgarian market, especially if these products are manufactured in countries with more permissive GMO policies.

The prevalence of GMOs in imported products stems from differences in agricultural practices, economic decisions, and supply chain dynamics in countries where GMOs are more widely used and accepted.

While the overall percentage of positive samples and the level of contamination is low, the spectrum of detected GM events is increasing, which also coincides with studies by colleagues from other countries (Waiblinger et al. 2023). The rise in the number of GM events has important implications for Bulgaria's regulatory system and market. It suggests that more types of GMOs are entering the market, which could make it harder for Bulgaria to effectively monitor and control GMO presence. Although Bulgaria follows EU regulations, the increase in GM events raises concerns about whether current systems can fully manage unauthorized GMOs. This could also affect consumer trust, especially for those who prefer non-GMO or organic products.

Components from GM plants not authorized in the EU were not detected which suggests that the Bulgarian market is in compliance with EU regulations regarding genetically modified organisms (GMOs). This aligns with expectations, as Bulgaria, like other EU member states, is required to adhere to strict EU policies on GMO imports and use. The absence of unauthorized GM components indicates effective monitoring and control systems in place, potentially reflecting Bulgaria's commitment to ensuring food safety and maintaining the integrity of its agricultural practices in line with EU standards. However, it may also suggest limited or no illegal trade in unauthorized GMOs within the country.

The assessment of the presence of GMOs in food samples on the Bulgarian market would help control laboratories and authorities for risk-based planning of food sampling in the future.

Conclusions

The analysis of food samples for genetically modified organisms (GMOs) has provided valuable insights into the current state of the market and the extent to which products comply with existing regulatory standards. The findings indicate that only 9% of the food products tested contained GMOs, all of which were below the threshold for mandatory labeling, meaning they are unlikely to pose any significant risk of consumer confusion or non-compliance with food labeling laws. In contrast, 91% of the samples tested showed no detectable GMOs, which suggests that genetically modified ingredients remain relatively rare in the food products surveyed.

Although the overall incidence of GMOs in the tested samples was low, a notable trend emerged. There was an increase in the variety of genetically modified events detected. This growing diversity reflects the continuous development of new GM technologies and the expansion of genetically modified crops and ingredients in the global market. Such a trend is consistent with findings from other European countries, as highlighted in a 2023 study by Waiblinger et al., which indicates that the scope of GM events is widening across the continent. This rise in the number of different GMOs detected could signal a shift in the agricultural landscape, where more GM products are being introduced and traded, potentially influencing future regulatory frameworks and consumer perceptions.

Additionally, the use of pre-prepared testing kits proved to be a practical and efficient tool for identifying GMOs in food products. These kits not only helped save time and reduce testing

costs but also demonstrated the potential for broader applications in food safety. Such systems could be extended to detect other food safety concerns, such as allergens offering a cost-effective way for food producers, regulators, and consumers to ensure the safety and integrity of the food supply.

Overall, the results underscore the importance of monitoring the presence of GMOs in food and maintaining robust testing and labeling practices. While current contamination levels remain low, the increasing diversity of GM events and the efficiency of modern testing systems suggest that both the regulatory landscape and consumer awareness around GMOs will continue to evolve. This could lead to future challenges and opportunities in terms of food safety, labeling policies, and public acceptance of genetically modified foods. The findings also suggest that ongoing investment in testing technologies and research into the broader impacts of GMOs will be crucial for ensuring transparency and maintaining consumer confidence in the food system.

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