УДК 577.21:631.523:604.6:664.012.1

STUDIES OF FOOD PRODUCTION AND RAW MATERIALS' CONTENT RELATIVELY TO GENETICALLY MODIFIED INGREDIENTS

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Aim. *Aim is to investigate presence of genetically modified (GM) ingredients of plant origin in food products and agricultural raw material presented on the domestic market of Ukraine.* ***Methods.*** *Detection of genetically engineered constructs was performed by using PCR in real time (Real-Time PCR).* ***Results.*** *Due to the fact that genetically modified organisms (GMOs) are ambiguously perceived by contemporary society, their use in foods is strictly controlled. Six-year results of the tests of food products and raw materials for GM of plant origin is presented.* ***Conclusions.*** *It is shown that with increasing awareness of consumers and producers of food products, and strengthening of state control over the use of GMOs their content in food products is reduced.*

Key words: *GMO, Real-Time PCR, food products and agricultural raw materials, monitoring.*

Introduction. Achievements of modern biological science are widely applied in many branches of human activity. Practice implementation of innovative biotechnological approaches allows purposeful changing of specie's genetic nature, creating of principal new plant forms, to increase yields, quality, adaptive potential. Transgenic technologies and GMO creating open new perspectives for production, health safety [1–3].

GMOs become gradually the realities of our life. They are actively involved into solution of many different problems such as quality improving of existing plant breed, insects protection, directed synthesis of pharmacological preparations, environmental cleaning from chemical pollutants etc [4]. From 1996 and to nowadays world areas of biotech crops in agrarian sector have been increased from 1,7 million hectares to 170 million hectares and present in 28 world countries [5, 6].

Although GM-crops have a range of advantages, the doubts concerning their safety exist [7, 8].

The factors of risk can be divided into three main groups. The first group includes risks concerning safety of GMO containing food for human health, such as toxic and allergenic properties, extention of pesticides accumulation, negative influence of the antibiotic resistance genes, other [9–11]. Ecological risks are connected with harmful influence of GM-plants to the environment. The examples

of this group of risks are harmful influence on biodiversity in agrocenosis, appearance of resistance to herbicides weeds and resistance to insecticides insects et al [12–15]. One more group of risk concerns social and economic consequences. It includes destruction of national system of seed production and dependence on import of seed commercially important crops, loss of the image of producer of ecologically clean products by country, etc. [16].

But for now we don't have enough scientific-based data of definite assessment about GMO safety. Thus in most world countries food products which are produced with GMO using, should be registered, post-register controlled and labeled [4, 5, 17, 18]. There is also a range of legislative regulation statements of circulation, transfers from abroad, treatment and using of biotech crops [19].

On the basis of state enterprise "UkrMetrTestStandard" scientific-production laboratory of molecular-genetic research for GMO analysis in food products and raw material was created. Laboratory is accredited by National Accreditation Agency for competence according to ДСТУ ISO / IEC 17025–21 requirements. Almost 10 000 samples were tested in laboratory from 2007 to 2013. Analysis of test results is given in this study.

Materials and methods

We used test-systems of our own production for GMO detection performing in food products and raw materials [20].

Total DNA was extracted by CTAB-precipitation method with own modifications [21]. Concentration of extracted DNA and purity by ratio A260/A280 and A260/A230 was detected by «BioPhotometer AG 22331» (Eppendorf, Germany).

Real-Time PCR-amplification was performed with iQCycler and CFX96 (BioRad). Reaction mix (volume–20 mkl) contained 100 ng of DNA, 10 мМ of Tris-HCl (pH 8,3), 50 мМ of KCl, 2,5 mM of MgCl2, 0,2 mM of dNTP mix, 5 pM of each primer, 2,5 pM of probe and 1 unit of Taq-polymerase (Thermo Scientific, Lithuania). We used olygonucleotide probes with FAM, JOE and ROX fluorescent dyes and BHQ1 and BHQ2 quenchers (Sintol, Russia and Metabion, Germany). Amplification consisted of initial denaturation during 3 min for 94 єС and following 45 cycles: denaturation – 20 sec for 95 єС, primer annealing and synthesis – 40 sec for 60 єС.

Detection of genetic constructs was performed for regulator elements (p35S, tNOS), target genes sequences (CP4 epsps, pat, bar) and transformation events (GTS40–3–2, MON810, RT73 et al) [22]. Quantitative GMO detection was performed by calibration curve with five standard points, according to ДСТУ ISO 21570:2008 requirements [23].

Results and discussion

The range of test-systems was designed in UkrMetrTestStandart according to legislative requirements during 2007–2013. Test-systems allow performing plant-derivative GMO content quantitative and qualitative analysis in food products and raw material, and identifying transgenic plant lines for transformation event. We've obtained technical conditions for test-systems in 2012 [20].

Designed test-systems are based on Real-Time PCR *TagMan* technology [24]. Reagents from "Sigma", "Fluka", "Thermo Scientific", "Metabion" and "Sintol" were used. Effectiveness of test-systems was assessed by certified reference material from IRMM, Belgium and by interlaboratory comparative rounds (JRC, European Union).

Laboratory performed GMO-content analysis of 413 food products and raw material samples of internal Ukrainian

market in 2007 (table 1). Agricultural raw material contained mainly such crops as soybean, maize, rapeseed, wheat, rice and products of their processing. In fact, quantity of analyzed samples was higher, because there is a practice of so called "joined samples" in laboratory, when from the very beginning of analyzing process samples of similar content of the same producer are joined into one sample. Data about such "joined samples" are registered only from 2010, quantity of performed analyses was given the same status as analyzed samples in this study. Results impressed. 21,8 % of analyzed samples contained GMO. Moreover, 36 food samples and 6 raw material samples contained GMO more than 0.9 %. Often GMOs were detected in sausage products and meat intermediate products which contained soybean or products of soybean processing. We found also GM-ingredients in confectionery and other food production which contained soybean and maize. Concerning agricultural raw material, it should be noticed that GM-positive samples were soybean, maize and rape crops samples. Thus the absence of GMO-control and circulation legislation has lead to unlawful delivery and growing of biotech crops, and using them in food production.

In 2008 number of tests and range of products were improved. Significant part of samples was food ingredients. This fact indicates the improvement of the producer's interest to the control of this indicator. These changes had reflection on the results of tests. Only 97 samples (8.2%) were GM-positive of 1177 samples. It should be notified that only 21 samples contained GMO, 13 of them contained GMO in concentration higher than 0.9 %. The situation with row materials was worse. GMO was detected in 76 samples and in 22 from them content was higher than 0.9 %.

In 2009, in connection with reception of Regulation of Cabinet Council of Ukraine № 468 from 13.05.2009 "About approval of the order of labeling a food products which contain genetically modified organisms or produced with their using and introduced" quantity of analysis significantly increased. General quantity of analyzed samples was 2126. GMO was detected in 107 samples, what amounts 5.0%. It specified by that fact for the first place the producers with products with-no GMO content took into account the necessity of GMO control. Also producers began to GMO-control the used raw material for their products. GMo was detected in 17 samples of food products, 13 of which contained GMO in quantity higher than 0.9 %. At the same time GMO was detected in 90 samples of raw materials and in 42 content was higher than 0.9 %

Quantity of analyzed samples in 2010 has been increased and amounted 2570. GMO was detected in 204 samples what amounts 7.9 %. Improvement was conditioned by the fact that the producers

Table 1. GMO monitoring of food products and raw material

Year	Sample quantity	GMO detected	Food products		Raw material	
			< 0.9%	> 0.9%	< 0.9%	> 0.9%
2007	413	90 (21,8%)	28	36	20	6
2008	1177	97 (8,2%)	8	13	54	22
2009	2126	107 (5,0%)	4	13	48	42
2010	2570	204 (7,9%)	62	9	102	31
2011	1866	59 (3,2%)	0	3	29	27
2012	2001	67 (3,3%)	5	3	25	34

of types of products which could contain GMO had understood necessity of control. Among the food products GMO was detected in 71 samples, from which only 9 contained GMO higher than 0.9 %. The situation with food raw materials became better too. Only in 31 samples of 133 GMO content was more than 0.9 %.

In 2011 quantity of analyzed samples decreased. It is hard to say what the reasons were. Perhaps it was because the net of GMO-test-laboratories increased in Ukraine. We analyzed 1866 samples, GMO was detected in 59 (3.2 %). GM-ingredients were detected only in 3 samples in quantity more than 0.9 %. Among the raw material, 56 GM-samples were detected, from which 27-with more than 0.9 % GM-content. This data is similar with data obtained in Russia after two years of total control of this parameter [25].

2001 food and raw-samples were tested during 2012. 67 samples were detected as GM-containing (8-food products and 59-raw material). GM-content higher than 0.9 % was for 3 food samples and for 34 raw material samples. General data for 2011 and 2012 do have a lot in common. Quantity GM-detected amounted 3.3%, considerably decreased quantity of GM-food samples (0.2 and 0.4 %, respectively) and decreased the quantity of raw material samples with GM-content (3.0 and 2.9 %, respectively) (figure 1).

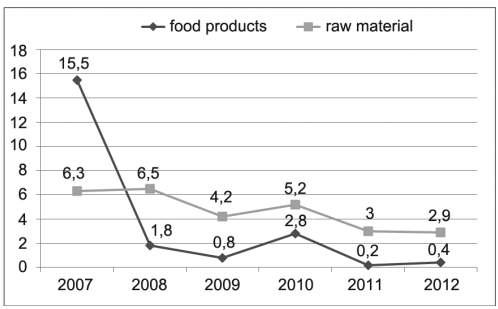

Figure 1. Test results for 2007–2012 and dynamic of GMO detection % in food products and raw material

The same tendency can be objected in 2013. Obtained data is under analysis for today and will be presented in the next publication.

As far as any GMO are prohibited in Ukraine, in most cases laboratories perform only screening analysis for regulation sequences p35S and tNOS and quantitative analysis for p35S. Identification of definite GM line, presence of target genes (CP4 epsps, pat, bar) and quantitative analysis for transformation event was performed in some cases due to consumer's will. As a rule-that consumers were major seed-traders, who export their production to EU countries. Among GM-soybean samples most of it was presented by GTS40–3–2 line, of maize – MON810, GA21, NK603 lines, rapeseed – RT73.

We consider obtained data reflects situation with GMO which we have in Ukraine for the last years.

Conclusions

For today our society has strictly determinate opinion concerning necessity of governmental regulation of GMO using and biotech crops-derivative products labeling. Thus there will be performing further control for biotech crops circulation in many countries. Ukraine, joining to Carthachena protocol in 2002, should also guarantee appropriate GMO control including circulation regulation, transfers abroad, treatment and using of biotech crops.

Study of food products and agricultural raw material which we have performed during six years, revealed presence biotech crops in Ukraine. This is GM-soybean, maize and rapeseed. In what way does GM raw material come to Ukrainian market? We didn't find the complete answer for this question so far. Partly this is result of non-controlled delivery GM seed in the past, when we didn't have legislative regulations

concerning GM crops rotation. Partly this is using of products of biotech crops processing from USA and Latin America. But in any case the situation is considerably changed for the last few years. GM-ingredients are almost absent in food products for today and agricultural raw material of plant origin is under strict control in more than 30 laboratories of Ukraine.

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Presented by B.V. Morgun
Received 28.03.14

ИССЛЕДОВАНИЕ ПИЩЕВОЙ ПРОДУКЦИИ И СЫРЬЯ НА СОДЕРЖАНИЕ ГЕНЕТИЧЕСКИ МОДИФИЦИРОВАННЫХ ИНГРЕДИЕНТОВ

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Цель. Исследовать наличие генетически модифицированных ингредиентов в пищевых продуктах и сельскохозяйственном сырье, представленном на внутреннем рынке Украины. **Методы.** Определение генно-инженерных конструктов проводили с использованием метода полимеразной цепной реакции в режиме реального времени (Real-Tima PCR). **Результаты.** В связи с тем, что генетически модифицированные организмы (ГМО) неоднозначно воспринимаются современным обществом, их использование в продуктах питания строго регламентируется. Представлены 6-летние результаты испытаний продуктов питания и сырья на наличие ГМО. **Выводы.** Показано, что с повышением уровня осведомленности потребителей и производителей пищевой продукции, а также повышением уровня государственного контроля использования ГМО в продуктах питания снижается.

Ключевые слова: ГМО, ПЦР в реальном времени, пищевая продукция и сельскохозяйственное сырье, мониторинг.

ДОСЛІДЖЕННЯ ХАРЧОВОЇ ПРОДУКЦІЇ ТА СИРОВИНИ ЩОДО ВМІСТУ ГЕНЕТИЧНО МОДИФІКОВАНИХ ІНГРЕДІЄНТІВ

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Мета. Дослідити наявність генетично модифікованих інгредієнтів у харчових продуктах та сільськогосподарській сировині представленій на внутрішньому ринку України. **Методи.** Визначення генно-інженерних конструктів проводили за використання методу полімеразної ланцюгової реакції у режимі реального часу (Real-Tima PCR). **Результати.** У зв'язку з тим, що генетично модифіковані організми (ГМО) неоднозначно сприймаються сучасним суспільством, їхнє використання в продуктах харчування строго контролюється. Представлено 6-річні результати випробувань продуктів харчування та сировини на наявність ГМО. **Висновки.** Показано, що з підвищенням поінформованості споживачів та виробників харчової продукції, а також посиленням державного контролю використання ГМО в продуктах харчування знижується.

Ключові слова: ГМО, ПЛР у реальному часі, харчова продукція та сільськогосподарська сировина, моніторинг.