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Investigation of the CaMV35SPromoter sequence in baby food and infant formula in the city of Tehran by Real-time PCR technique

Beheshti zadeh, R. [1], Safaeian, Sh. [2*], Moslemi, E. [3], Mosavi nadoshen, R.[2] , Esfahani, K. [4]

1. Department of Food science, Islamic Azad University, North Tehran Branch, Tehran, Iran.
2. Department of Food science, Islamic Azad University, North Tehran Branch, Tehran, Iran.
3. Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran.
4. Department of Plant Bioproducts, Institute of Agricultural Biotechnology (IAB), National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

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*Corresponding Author E-Mail:
shila2462462@yahoo.co.in

ABSTRACT

Corn and soybeans have the largest area of GM crops in the world. Milk powder and baby food are processed foods that contain corn and soybeans,therefore, monitoring transgenic corn and soy in processed food has been investigated in several studies.In this study, 40 samples of baby food and milk powder were collected from pharmacies and supermarkets in Tehran. All the samples were extracted using Azmaelixir DNA kit. Internal control genes for soy (Lectin) and corn (Zein) were checked to ensure the extraction. Then, the presence of CaMV35Stransgenic Promoter was checked by Real-time PCR technique. The results showed the presence of this sequence in 2.5% of baby food samples and 10% of samples in infant formula. The preliminary results of this study suggest more investigation and monitoring is necessary for the possible labeling of these products.

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1-Introduction

With the increase in population and the need for more food, ensuring food security and knowing the components of food products has become one of the most challenging issues all over the world. to pests and environmental stresses through the production of plants[1]GMO takes place[1]. Lion Infant formulas are nutritious reconstituted liquids or powders for infants and young children that work as substitutes for human milk. Formulas play an important role in the diet of infants, as they are the only source of nutrients for some infants[2].

Dry milk is mainly composed of lactose, corn maltodextrin, hydrolyzed minerals and protein. Milk fat includes palm olein, soybean oil, corn oil and sunflower oil[3]. Maize is considered the most consumed grain in the world and in terms of production, it ranks third after soybeans and wheat. Corn used in the food industry can be resistant to herbicides and express a protein from bacteria*Bacillus thuringiensis* which acts against certain pests is genetically modified. About 80% of soybeans cultivated in the world are genetically modified[4]. Soybean is the flagship of transgenic products and with 47% of the land under cultivation of transgenic products, it occupies the first place among crops resulting from modern biotechnology. Diagnosis based on DNA is very accurate and sensitive and can be quantified.[5]. The CaMV35S promoter from the cauliflower mosaic virus is one of the most widely used promoters of transgenic plants, and considering that this gene is present in most commercial transgenic products, it is one of the most important options for identifying transgenic agricultural products.[3].

Although the labeling of transgenic products is not mandatory by many countries, most of the countries that consume and produce transgenic products perform qualitative and quantitative tests to identify genetically modified products based on national and international laws.[2].

The most common methods of detecting GMO based on DNA is the PCR method, which can be detected even with small amounts. In Real-time PCR technique, it is also possible to quantitatively measure the amount of genetic modification products in the investigated samples[6].

In the present study, the presence of CaMV35S precursor in milk powders and baby foods available in pharmacies in Tehran province has been investigated by Real-time PCR technique.

2- Materials and methods

2-1-Extraction of genomic DNA and checking its quality

First, 40 samples (20 milk powder, 20 baby food) were prepared from pharmacies in Tehran. Then, genomic DNA extraction was performed with a DNA extraction kit (Azma Elixir Research). Also Mon810[2] CRM as a control was prepared from Urfin Company. The amount and quality of the extracted DNA was measured with a Nanodrop device (Thermo Fisher Scientific) made in the United States at 260 and 280 wavelengths.[7].

2-2-primers used

The primers used for CaMV35S promoter, soybean internal control gene (*Lectin*) and internal control gene of maize (*which*) Are. After checking the target sequences on the NCBI website, the primers were designed by Primer Express program and their accuracy was checked by Gene Runner and BLSTN software.[7]. The sequence of primers used can be seen in Table 1 below.

1. Genetically Modified Organism

2. Certified Reference Materials

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Table 1 Oligonucleotide Primers Used in Real-time PCR

Target gene	First	Sequence (5'-3')	Product size (bp)	Reference
Lectin(Soy)	Lectin-F Lectin-R	GACGCTATTGTGACCTCCTC GAAAGTGTCAAGCTTAACAGCGACG	110 bp	**[3]**
CaMV35S	F R	GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA	195 bp	**[3]**
which(Maize)	F R	AGTGCGACCCATATTCCAG GACATTGTGGCATCATCATTT	139 bp	**[3]**

2-3- Real-time PCR

PCR reaction was performed on the transgenic samples (CRM5, 1, 0.1) percent and also for lectin (soybean internal control), zein (corn internal control) and CaMV35S gene sequences in StepOne (ABI) machine. The reaction components include: 10µl mastermix, 0.5µl primers (F and R), 1µl of sample DNA and 8.5µl water were added to reach a volume of 20µl. The reaction was performed at a denaturation temperature of 95°C for one minute and annealing temperature of 60°C for 30 seconds and also at an extension temperature of 72°C for 30 seconds with 30 repetitions. Reactions were performed in triplicate. from CRM sample as positive control for CaMV35S and internal control (*which*, *Lectin*) was used to track corn and soybeans. Also, water without DNA was used as a negative control to confirm the absence of contamination[5].

3. Results and Discussion

3-1- Extraction result

The quality of extracted DNA was checked by nanodrop. The concentration of all samples was in the range of 116 to 190 ng of DNA per microliter and the absorption ratio of our extracted samples was between 1.7-2.[8].

2-3-Real-time PCR results and discussion on the obtained results

There are ingredients such as corn and soy in baby formula and food. It is very important to use the appropriate protocol to accurately evaluate the transgenicity of these products. As can be seen in Figure (1), the melting curve for the internal control gene of corn is reproduced well, which indicates the presence of corn in all samples. is. Also, in figure number (2) the internal control gene of soybean has been reproduced. Reproduction of the internal control gene of soybean and corn (Zein and*Lectin*) in all extracted samples of powdered milk and baby food, it is a proof of correct extraction and the presence of soy and corn in the samples.[11].

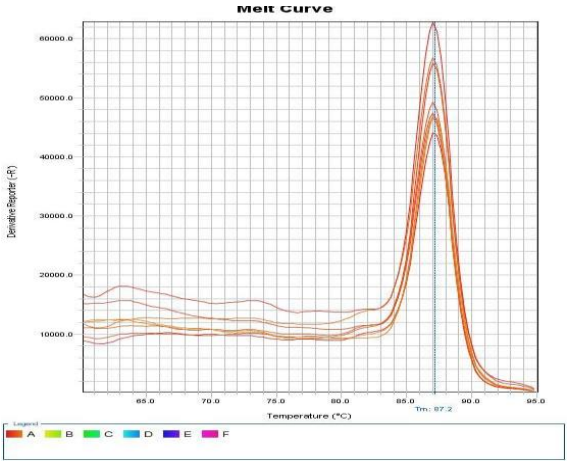

Fig 1 Melt curves of Zein showed specific PCR product

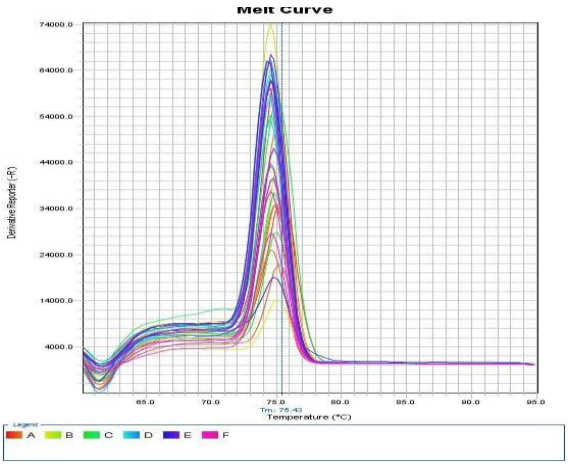

Fig 2 Melt curves of lectin showed specific PCR product

In order to produce transgenic corn, elements such as NOS:, CaMV35S and nptII have been widely used, and in this research, CaMV 35S promoter sequence was investigated [9].SourceThis sequence is the cauliflower mosaic virus and is present in most commercial transgenic products, for this reason it is one of the most important options for screening transgenic

products [9]. In a research to investigate the transgenic status of rice seeds, specific primers for CaMV 35S have been used [12].

In this research, the presence of transgenic processed soybeans and corn was observed in a limited number of samples. The previous results indicate a high percentage of imported transgenic soybeans and corn [11]. In another study in Mozambique, CaMV35S and Nos sequences were detected in 22 of 47 samples. While in this research, 2 samples of baby food and 3 samples of dry milk were found to carry CaMV35S [14].

Also, the presence of transgenic corn in processed foods that are commercially available in Iran is confirmed by CaMV35S primer [12]. In 2020, Bara et al. still reported the two sequences of CaMV35S and Nos terminator as the most important elements in transgenic events. [14].

Real-time PCR test results for the samples in which the CaMV 35S sequence was detected, as well as the Ct of the detected samples are shown in Table 2.

Table 2.DNA Concentration and Real-time PCR of Samples

sample	DNA concentration (of/ µl)	*CaMV 35S*geneCT values
Infant formula	180.5	29.48
Infant formula	176.8	30.70
Infant formula	165.8	31.92
Baby food	134.9	29.95
Baby food	190.7	30.36

Figure 3 shows the melting curve for CaMV35S sequence in 3 milk powder samples and 2 baby food samples.

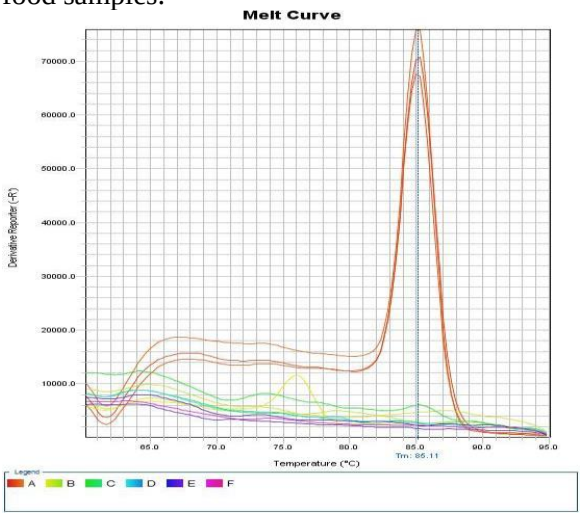

Fig 3 Melt curves of CaMV35S

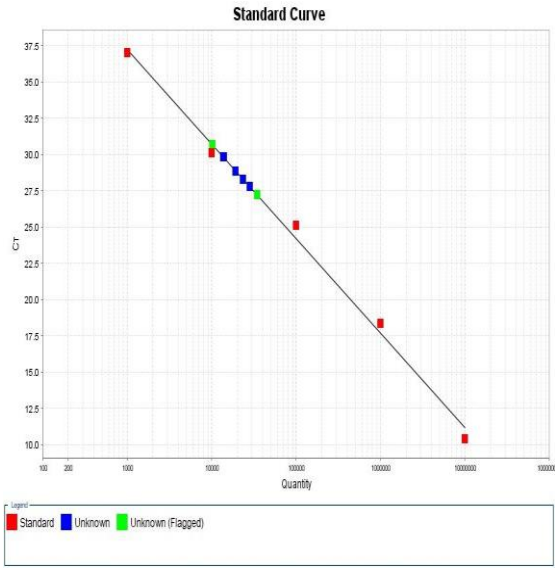

Fig 4 Standard curve for CaMV35S

In the standard chart, the vertical axis (Ct) and the horizontal axis shows the quantity. As can be seen in figure (4), to determine the sensitivity of the test, 1000 copies of the number can be identified, while in a similar study, at least 10000 copies They traced the number [11] also in the standard curve, the Ct factors show that this value has an inverse relationship with the concentration and the Ct value increased with the decrease of the concentration. were able to identify 20 copies of numbers, while in this research 1000 copies of numbers could be identified [17].

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Table 3 Identification of CaMV35S, Lectin, Zein Sequences by Real-time PCR in Baby food and Infant Formula

Sample type	Number of samples	CaMV35S positive	Endogenous gene	Endogenous gene	Negative
			Lectin	which	
Baby food (Iranian)	10	0	10	10	10
Baby food (imported)	10	2	10	10	8
Infant formula (Iranian)	10	1	10	10	9
Infant formula (imported)	10	2	10	10	8
Total	40	12.5%	100%	100%	87.5%

As can be seen in Table No. 3, CaMV 35S sequence was detected in 12.5% of the baby food and milk powder samples examined, of which 2 are imported milk powder and 1 is Iranian milk powder. In a similar research in 2020 and using the Real-time PCR technique by means of a probe in Algeria, the presence of transgenic products was not detected [16].

4 - Conclusion

The results of this study show the detection of CaMV35S sequence in 12.5% of milk powder and baby food available in Tehran market. According to the existing laws regarding the use of transgenic products, the need to label the said products and review these products by regulatory bodies is suggested.

5-Resources

[1] Chawla, H., 2011. Introduction to plant biotechnology (3/e). CRC Press.

[2] Hediger, M.L., Overpeck, M.D., Ruan, W.J. and Troendle, J.F., 2000. Early infant feeding and growth status of US-born infants and children aged 4–71 mo: analyses from the third National Health and Nutrition Examination Survey, 1988–1994. *The American journal of clinical nutrition*, *72*(1), pp.159-167.

[3] Pickett-Bernard, D., 2006. Infant Formula: Evaluating the Safety of New Ingredients. Journal of Human Lactation, 22(2), pp.231-232.

[4] Berdichevets, I.N., Shimshilashvili, H.R., Gerasymenko, I.M., Sindarovska, Y.R., Sheludko, Y.V. and Goldenkova-Pavlova, I.V., 2010. Multiplex PCR assay for detection of recombinant genes encoding fatty acid desaturases fused with lichenase reporter protein in GM plants. Analytical and bioanalytical chemistry, 397(6), pp.2289-2293.

[5] Arun, Ö.Ö., Yılmaz, F. and Muratoğlu, K., 2013. PCR detection of genetically modified maize and soy in mildly and highly processed foods. Food Control, 32(2), pp.525-531.

[6] Marmiroli , N. , Maestri , E. , Gulli , M. , Malcevschi , A. , Peano , C. , Bordoni , R. and De Bellis , G. , 2008 . Analytical and Bioanalytical Chemistry, 392(3), pp.369-384.

[7] Gryson, N., 2010. Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review. Analytical and Bioanalytical chemistry, 396(6), pp.2003-2022.

[8] Zhang, Q., Maroof, M.S., Lu, T.Y. and Shen, B.Z., 1992. Genetic diversity and differentiation of indica and japonica rice detected by RFLP analysis. *Theoretical and Applied Genetics*, *83*(4), pp.495-499.

[9] Ahmed, F.E., 2002. Detection of genetically modified organisms in foods. TRENDS in Biotechnology, 20(5), pp.215-223.

[10] Vráblík, A., Hodek, J., Demnerová, K., Soukup, J. and Ovesná, J., 2012. Development and verification of PCR based assay to detect and quantify garden pea lec gene. Czech Journal of Food Sciences, 30(3), pp.247-257.

[11] Safaei, P., Rezaie, S., Alimohammadi, M., Agha Kuchak Afshari, S., Mehdizadeh, M. and Molaee Aghaee, E., 2020. Qualitative PCR-based detection of genetically modified soy and

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maize products in Iran. *International Journal of Food Properties*, *23*(1), pp.459-469.

[12] Ashrafi-Dehkordi E, Maghsoudi K, Babajafari S, Mazloomi M. Investigation on transgenic rice status in Shiraz consumer market using PCR. Genetic Engineering and Biosafety Journal. 2020; 9 (2) :204-212

[13] Ashrafi-Dehkordi, E., Mazloomi, S.M. and Hemmati, F., 2021. A comparison of DNA extraction methods and PCR-based detection of GMO in textured soy protein. *Journal of Consumer Protection and Food Safety*, *16*(1), pp.51-57.

[14] Joelma, L.B., Olvia, C.P., Amlia, M.M., Artimsia, M.M., Lucinda, D.A., Lus, D.N. and Dcia, C., 2022. Detection of genetically modified organisms in food products commercialized in Mozambique. *African Journal of Food Science*, *16*(2), pp.22-29.

[15] Rabiei, M., Mehdizadeh, M., Rastegar, H., Vahidi, H. and Alebouyeh, M., 2013. Detection of genetically modified maize in processed foods sold commercially in Iran by qualitative PCR. *Iranian journal of pharmaceutical research: IJPR*, *12*(1), p.25.

[16] Brara, Z., Costa, J., Villa, C., Grazina, L., Bitam, A. and Mafra, I., 2020. Surveying genetically modified maize in foods marketed in Algeria. *Food Control*, *109*, p.106928.

[17] Cottenet, G., Blancpain, C., Sonnard, V. and Chuah, P.F., 2019. Two FAST multiplex real-time PCR reactions to assess the presence of genetically modified organisms in food. *Food chemistry*, *274*, pp.760-765.

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مقاله علمی_پژوهشی

بررسی حضور ژن تراریخته *CaMV35S* در شیر خشک و غذای کودک موجود در تهران با تکنیک Real-time PCR

رامین بهشتی زاده[۱]، شیلا صفاییان[۲*]، الهام مسلمی[۳]، رضوان موسوی ندوشن[۲]، کسری اصفهانی[٤]

۱-دانشجوی دکتری تخصصی، گروه علوم و صنایع غذایی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران.

۲-استادیار گروه علوم و صنایع غذایی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران.

۳-استادیار گروه زیست شناسی، واحد تهران شرق، دانشگاه آزاد اسلامی، تهران، ایران.

٤-استادیار، گروه زیست فرآورده های گیاهی، پژوهشکده بیوتکنولوژی کشاورزی، پژوهشگاه ملی مهندسی ژنتیک و زیست فناوری، تهران، ایران.

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* مسئول مکاتبات:

shila2462462@yahoo.co.in

چکیده

ذرت و سویا بیشترین سطح کشت تراریختگی را در دنیا به خود اختصاص داده اند. با توجه به افزایش واردات سویا و ذرت به ایران و همچنین به دلیل استفاده از روغن های محصولات استراتژی در شیر خشک و غذای کودک، لذا ردیابی تراریختگی در مواد غذایی فرآوری شده با تکنیک Real-time PCR یک نیاز اساسی می باشد. ابتدا ٤٠ نمونه غذای کودک و شیر خشک از داروخانه ها و سوپر مارکت های شهر تهران جمع آوری شد. همه نمونه با استفاده از کیت DNA آزما اکسیر پژوه استخراج شدند و بررسی کمی غلظت اسید نوکلئیک با دستگاه نانودراپ انجام شد. سپس برای بررسی کیفی استخراج DNA انجام شده، تست PCRژن های کنترل داخلی برای سویا (*Lectin*) و ذرت(*Zein*) گذاشته شدند. سپس با تکنیک Real-time PCR حضور ژن تراریخته *CaMV35S* بررسی گردید و نتایج به دست آمده از حضور ژن تراریخته در ۲.۵ ٪ نمونه غذای کودک و ۱۰٪ نمونه در شیر خشک را نشان دادند. بنابراین در این مقاله میزان نفوذ ژن تراریخته در غذای کودک و شیر خشک مورد بررسی قرار گرفته است.