DETECTION OF GENETICALLY MODIFIED FOODS: COMPARISON OF (DNA) EXTRACTION METHODS FROM GENETICALLY MODIFIED MAIZE AND ITS DERIVED PRODUCTS.

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ABSTRACT

This work was conducted to compare between six methods for the extraction of DNA from raw maize and its derived products. On the other hand, the method (s) that given the highest levels of DNA yields and quality will be chosen to screen and detect the genetic modification in the samples that collected from the Egyptian food market. The methods were evaluated for the extraction of DNA from maize kernels (no treatment), maize flour (mechanical treatment), maize snacks, canned maize (sweet corn), frozen maize (sweet corn), maize starch, maize extruded, popcorn, corn flacks, (mechanical, thermal and treatment). Maize snacks, corn flacks, bread with corn flour and maize starch. The quality and quantity of DNA extracted from standards, containing known percentages of GMO material and from different food products. GMO Screen 35S/Nos test kit for qualitative detection of GMO varieties in food, feed and seed was used to screen the genetic modification in the samples. The positive samples for 35S promoter and or NOS terminator were identified by standard methods that adopted by EU. All methods extracted a good DNA quality from raw materials for most of the raw materials. High pure DNA extraction kit recovered the highest levels of DNA. DNA yields for maize-derived foods generally decreased with the extent that the product had been processed. The High pure DNA extraction kits (Roch) was generally the best method for the extraction of DNA from most of the maize-derived foods. The results from screening indicated that 17 samples from investigated samples were positive for the presence of 35S promoter. 34% from the samples positive for the genetically modified maize line Bt 176. Keywords: GMO, DNA extraction, Screening, maize, PCR, High pure GMO

INTRODUCTION

After the approval and cultivation of various genetically modified crops in united states and Europe in recent years, nucleic acid have become an important tool in food analysis (Peano et al., 2004 & Smith et al., 2005)

Several identification methods were developed. These methods were classified into different groups which focusing DNA, proteins, or other specific analysis (Gachet et al., 1999, Ahmed, 2002, Elsanhoty et al., 2006). Most of official identification method, which focused on the detection of the genetically modified foods depended on DNA. Polymerase chain reaction (PCR), was applied to identify the 35S promoter of the modified gene in Round up Ready soy bean. A specific system was then applied to the identification of the specific gene, epsps, gene of 5-enolpyruvyl-shikimate-3-phosphate synthesis

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(EPSPS), for samples with 35S positive PCR results. For samples with 35S negative PCR results, the DNA check system was applied to identify the soybean's specific lectin gene. No soybean was identified in the samples if negative results were obtained. A specific system was then conducted for those samples with positive results. When the result was positive, RRS was identified; when negative, non-RRS soybean was identified (Eisanhoty et al., 2005). Since the PCR method bases on DNA, when DNA level is low, DNA integration is low. As the result, the present PCR inhibitors are hard to be separated and the accuracy and sensitivity of PCR methods will be dramatically interfered. This concern happens quite often in many processed foods, e.g. salad oil, fine soybean lecithin, starch extractants (Elke et al., 2002).

European Union consumers are mostly against the use of genetic engineering in the agro food sector. According to the new European Community regulations on genetically modified food and feed, it is necessary to label food products when the concentration of the genetically modified material is higher than 0.9% (Vodret et al., 2007). Compulsory labeling and the introduction of new transgenic events highlight the need to develop new analytical methods to quantify the different genetic modified organisms in food. The quantitative real time Polymerase Chain Reaction is currently the main technique used for this purpose and the extraction of DNA from food samples represents the first step for its application. A large number of protocols are currently available: in addition to the standard laboratory techniques, other procedures based on specific kits with faster extraction times can be applied. One of the problems to perform most of the proposed method is the quantity and quality of the DNA extracted from genetically food and their derived products. It has been shown that DNA suffers degradation due to the thermal treatment to which the genetically modified foods are subjected during the canning process (cooking and sterilization) but also the type of liquid that is added may play a role in this degradation (Bauer et al., 2003). Genetically modified derived products may present different liquid media, like brine, oil, vinegar or tomato and other food ingredients these may produce differences in the quantity and quality of the extracted DNA and lead to severely reduce amplification efficiency in PCR or may render target sequences undetectable, fat, salts, acid and other additives in food matrix may also contribute to PCR inhibition (Terry et al., 2002). Many DNA extraction protocols are available but they have been rarely compared (Olexova et al., 2004; Peano et al., 2004 and Chapela et al., 2007). The objective of this work was to analyze the effect of the different treatment and willing media in the quantity and quality of the extracted DNA, and the efficiency of different commercial DNA extraction methods from genetically modified maize and some of its derived products. The DNA extraction methods were evaluated for the amount of genomic DNA extraction, the degradation of the DNA extracted, and the effect of DNA on PCR performance and the ability to estimate if the quantity of GM maize lower than 1% in different processed foods in the Egyptian food market.

MATERIALS AND METHODS

Food samples and reference standard:

Certified reference material (CRMs), produced by the institute for reference material and measurements (Geel, Belgium) were used as negative and positive controls for maize line Bt176. CRMs was purchased from Fluka. Fifty food samples used for this experiment which derived from maize or contain it as ingredient. The samples were collected from the Egyptian food market. The samples are listed in Table 1.

Table 1. Samples used to test the performance of extraction methods

Samples	Number	· · · · · · · · · · · · · · · · · · ·	Number of	Presence	
Odulhida	of	processing	samples	of 35S	samples
	samples		positive for	promoter	, ,
!	Samples	}	invertase	promoter	Bt malze 176
			gene	1	Dimarze 110
			Ague		
Certified Reference Material	2	Low proceed	2	2	2
(Institute For Reference Materials and Measurements)	_				
Maize Kernel (yellow maize USA)	5	raw	5	4	4
Egyptian White maize	3	raw	3	3	3
Com flour	2	Low processed	2	2	2
Maize snacks	5	Highly	5	2	2
		processed		1	
Canned maize (sweet corn)	5	Highly	5	3	3
Constant		processed		2	
Frozen maize (sweet com)	5	Highty processed	5	2	2
Maize Starch	5	Highly	5	-	-
		processed			
Maize extruded	5	Highly processed	5	-	•
Popcorn	5	Highly	5		
Popositi	3	processed	3		•
Corn flacks	5	Highly	5	1	1
	1	processed	_	1	1
Bread with corn flour toritale	5	Highly	50	17	17
		processed		 	ļ <u>. </u>
Total samples	50		50	17	17

Samples pre-treatment:

The samples that contain oil and lipid were treated to remove the oil and lipid. The samples were soaked in chloroform :methanol :water (1:2:0.8) overnight. The defatted samples was recovered by filtration and stored frozen until DAN will be extracted.

DNA extraction and purification:

Samples were homogenized with a mixer; then the DNA was extracted using six different techniques: 1-CTAB extraction (Anonymus, 2002); 2-Genome R DNA Isolation kit (Qbiogene), 3- High pure GMO Sample Preparation Kit, Roche Diagnostics, 4-Nucleospin (Clontech), Genomic Prep (Amersham Pharmacia Biotech, Italy), 5- DNA extraction kits from plant inveterogene (USA), pure link TM plant total DNA purification kit for

purification of DNA from plant, 6- Plant genomic DNA extraction from V gene Biotechnology limited. DNA was extracted by different kits according to producers instructions. All types of products and certified reference material were extracted in diplicate. The extracted DNA was stored at -20 °C until using for subsequent steps.

Quantification of genomic DNA:

Spectrophotometric method. Spectrophotometric optical densities of 260 nm and 280 nm were used to investigate the DNA quantity (Sambrock et al., & Maniatis, 1989). DNA purity was measured using the appropriate ratio of OD260: OD280 (1.65-1.85). Concentrations (ng/µl) and A260/A280 readings were recorded for each sample. The extracted DNA concentration was measured and adjusted by dilution to conc. 20-25 ng/µl prior to PCR, using bi-distilled, deionized, sterile water (Fluka, Germany).

Oligonucleotide primers

Primers used in this section of study together with their target specific part of the investigated DNA are listed in Table (2). All primers were synthesized by Eurofins MWG, Germany and obtained in a lyophilized state. All primers were solved before use to obtain a final concentration of 20 pmol/µl of each.

Table 2. The Primers used for amplification of DNA extracts

Primer	Sequence 5'- 3'	Target element	Fragment length	References
IVR1-F/ IVR1-R	5'- CCg CTg TAT CAC AAg ggC Tgg TAC C-3' 5'- ggA gCC CgT gTA gAg CAT gAC gAT C-3'	Maize Invertase gene.	226 bp	Ehlers et al., (1997)
Cry03 / Cry04	5' - CTC TCg CCg TTC ATg TCC gT -3' 5' - ggT CAg gCT CAg gCT gAT gT -3'	Transition site from the CCDPK-promoter into the amino terminal sequence of synthetic Cry1A(b) gene in Bt 176 maize.	211 bp	Anonymus (2002)

GMO screen 35S/NOS

The extracted DNA from the samples were screened for the 35S/NOS by using of test kit for qualitative detection of GMO varieties in food and feeds (GeneScan- Germany Cat nos.: 5221102210) according to producer's instructions.

DNA amplification and PCR condition

PCR was carried out on a Gene Amp PCR system 2400 (Perkin Elmer, Germany). For each series, a master mix was prepared. Each PCR reaction mix had 25 μ l total volume and contained 2.5 μ l PCR buffer (10 x concentrate, Perkin Elmer), 2 μ l MgCl₂ solution (25 mM), 1 μ l dNTPs solution (0.2 mM each of dATP, dCTP, dGTP and dTTP), 0.5 μ M of each primer, 1 Unit AmpliTaq Gold polymerase (Perkin Elmer), 2 μ l of template extracted DNA and was completed to 25 μ l with water. Table (3) explains the time/temperature profiles used in PCR for each primer pair. On the other

hand, PCR was done for screening of 35S/NOS according to producers instructions. All amplicons were stored at 4 °C until gel electrophoresis.

Table (3) Time / temperature profiles for qualitative PCR with DNA extracted from maize samples using the primer pairs

described in Table (2) Primer pair Initial Denaturatio Annealing Extension Cycles Final denaturation elongation IVR1-F /IVR1 42 12 min. at 30 sec. at 30 sec. at 30 sec. at 10 min. at72°C 95°C 72°C 95°C 64°C Cry03 /Cry04 12 min. at 30 sec. at 30 sec. at 38 10min, at 72°C 30 sec. at 95°C 95°C 63°C 72°C

Table 4. A260/ A280 ratios of DNA extracted from investigation DNA extraction methods.

- 67	Hatti	on meanous.					
	СТАВ	Commercial DNA extraction kits					
Samples		pure link [™] plant total DNA purification kit for purification of DNA from plant (invitrogene™)	GMO Sample Preparation	Genome ^k DNA Isolation kit (Qbiogene)	DNA extraction from V gene Biotechnology limited	Genomic DNA Extraction Kit for Food Samples (Cartagen	
Certifièd Reference Material (positive control)	1.92	1.91	2.11	2.01	1.92	2.08	
Maize Kernel	1.83	1.90	2.09	2.01	1.96	2.02	
Com flour	1.86	1.88	2.10	1.98	1.89	1.99	
Maize snacks	1.58	1.73	1.89	1.83	1.8	1.87	
Canned maize (sweet com)	1.76	1.82	1.92	1.84	1.86	1.85	
Frozen maize (sweet com)	1.82	1.86	1.94	1.87	1.81	1.82	
Maize Starch	1.54	1.70	1.80	1.69	1.65	1.67	
Maize extruded	1.61	1.68	1.89	1.71	1.74	1.75	
Popcom	1.55	1.69	1.88	1.73	1.70	1.77	
Corn flacks	1.66	1.59	1.74	1.70	1.65	1.72	
Bread with corn	1.54	1.61	1.74	1.70	1.65	1.74	

Agarose gel electrophoresis examination.

To confirm the existence of sufficient DNA to produce banding in every specimen tested, agarose gel electrophoresis was employed. Quality and quantity characteristics of the extracted DNA were further checked by electrophoresis on 1% (w/v) agarose gel (TAE buffer system) and ethidium bromide staining (0.5 μg/ml). The results were visualized on a UV transillumination (254 nm) with a and documented using Fluorchem Imager 5500 system (Alpha Innotech, USA). Agarose gel preparation as well as electrophoresis were carried out using Tris-base/borate (TBE) buffer solution (pH 8.0), containing 45 mmol/L Tris-base / boric acid and 1 mmol/L EDTA adjusted with hydrochloric acid. To determine the size of the DNA fragments, DNA of known size (100 bp DNA marker, Gibco BRL, USA) together with the different amplicons were separated on 2% w/v agarose gel (LE, Roche)/TBE

buffer stained with 0.01% ethidium bromide solution (0.5 mg/L). 10 μ l of all amplicons and DNA marker were stained before gel electrophoresis by 2 μ l xylenecyanol dye solution (1 mg xylenecyanol, 400 mg sucrose and completed to 1 ml with water), and then subjected to electrophoresis for 45 min. The amplicons were made visible by ethidium bromide staining and documented using Fluorchem Imager 5500 system (Alpha Innotech, USA).

RESULTS AND DISCUSSION

DNA extraction, concentration, purity and fragmentation state:

DNA was extracted from foodstuffs chosen on the basis of the complexity of their composition and technology treatment. Foodstuffs containing maize ingredients used in this study were described before in the materials and methods (Table 1) and the samples were the following: maize kernels (no treatment), maize flour (mechanical treatment), maize snacks, canned maize (sweet corn), frozen maize (sweet corn), maize starch, maize extruded, popcorn, corn flacks, bread with corn flour (mechanical, thermal and treatment. Maize snacks, corn flacks, and maize starch, moreover, contain ingredients capable of inhibiting PCR such as fatty acid and oils. DNA was extracted with the six methods and the quantity of obtained DNA was evaluated by using spectrophotometer and electrophoresis. The quantification of DNA on agarose was achieved with all samples and different DNA extraction to detect the band corresponding to the genomic DNA (Figure 1)

The data obtained from figure 1 indicated that there were differences in DNA obtained from the foodstuffs that (mechanical, thermal and treatment) by using different methods for extraction. It was impossible to perform genomic quantification on agarose gel except for the samples that used the High pure GMO Sample preparation Kit, Roche gave the highest yield of DNA from complex foodstuffs such as maize snacks, canned maize (sweet corn), frozen maize (sweet corn), maize starch, maize extruded, popcorn corn flacks and bread with corn flour. The quality of the DNA extracted from food samples is generally influenced by these factors: the grade of damage (e.g., depurination) of the DNA, the presence of PCR inhibitors in food matrices; and the average fragment length of the DNA extracted. These factors are dependent on the samples itself, the processes carried out during the production of the food, physical and chemical parameters of extraction method utilized (Peano et al., 2004). The exposure to heat is known to cause fragmentation of high molecular weight DNA (Hupfer et al., 1998 & Toyota et al., 2006), and physical and chemical treatments will cause random breaks in DNA strands, thus reducing the average DNA fragment size. Many foods, such as vegetables and fruits are characterized by their acidity, thus accelerating the acid-catalyzed reactions in course in thermal treatments. On the other hand, processing at alkaline pH is part of the production of other foods; a typical example is use of strong alkaline and or acidity solution in the initial stages of the prepration of bread, starch and other similar foods from maize. The DNA is very sensitive to acid and alkaline agents because of mechanism of hydrolytic degradation of DNA. At acid pH, purines are removed from the nucleic backbone due to the cleavage of N-glycosidic bounds between deoxribose residues and bases.

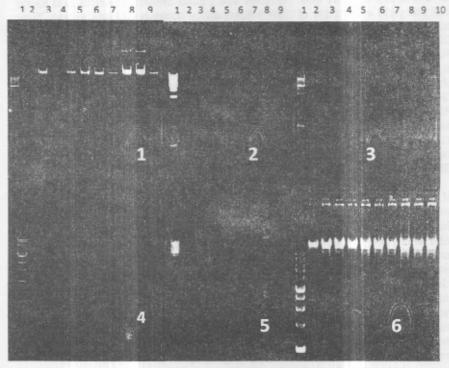


Figure 1. Agrose gels of total DNA extracted from 1-Plant genomic DNA extraction from V gene biotechnology limited, 2-Nucleospin (Clontech), GenomicPrep (Amersham Pharmacia Biotech, Italy), 3- Genome^R DNA Isolation kit (Qbiogene), 4- CTAB method, 5- DNA extraction kits from plant inveterogene (USA), 6- High pure GMO Sample Preparation Kit, Roche Diagnostics. Lane 1: M 1 Kbp. Line 2: DNA extracted from bread with corn flour toritale.; Lanes: 4: DNA from maize kernals from USA; lane 5: DNA from maize flour; lane 6: DNA from cannend maize); lane 7: DNA from frozen maize (sweet corn); lane 8: DNA from starch; lane 9: DNA from extruded maize; line 10: DNA from Popcorn; line 11: corn flacks, respectively in 1, 2, 3, 4, 5 and 6.

Subsequently, adjacent 3', 5'-phosphodiester linkage are hydrolyzed, leading to the shortening of DNA strands (Anklam et al., 2002 & Yamaguchi et al., 2003). The results indicated that good results were obtained with the High

Pure GMO sample preparation Kit Roche diagnostics. DNA extraction with High pure GMO Sample Preparation Kit, Roche diagnostics was given higher concentrations when compared to the other extraction methods, and the purities were very good. Similar results were obtained by Smith et al., (2005), Yohimitsu & Hori (2003) and Sisea & Pamfi (2007) who compared methods for extraction of DNA from potatoes and potato derived products and found that the yield and quality of DNA influenced by cooking and processing. They concluded that Wizard method was the best methods for the extraction of DNA from most potato-derived foods and Maxwell™ 16 Tissue DNA Purification Kit is best suited for raw or low processed matrices such as seeds and flour and CTAB methods not suitable. On the other hand, the obtained results were not agreement with those obtained by Milia et al., (2008) who used three different extraction methods for detecting Roundup Ready soybean in processed food from Italian market, and found that all the methods that used (CTAB, Kit PrepmanTM Ultra, ABI PRISM 6100) were suitable to isolate the DNA from Processed food and that in all the analyzed samples the quantity of RRS was less than 0.9%.

GMO Screen 35S/NOS:

Depending on the results obtained from the quantification and purity of DNA. All extracted DNA from the samples by high pure GMO sample preparation Kit Roche diagnostics were chosen to screen by using GMO Screen 35S/NOS test kit for qualitative detection of GMO varieties in food, feed and seed according to manufactures instruments. The control reaction (chloroplast DNA) was done as indicate whether DNA of sufficient quantity and quality has been isolated from the samples. A specific DNA sequence of 199 bp in length from chloroplast gene was amplified both from conventional plant DNA and from genetically modified plant DNA. The GMO PCR indicates the presence of genetically modified DNA (data not shown). The amplicon was specific for a GMO specific genetic elements (35S promoter or NOS terminator). 17 samples were positive results from GM) screen 35S/NOS at the expected amplicon size of 123 bp (Figure 2)

materials with six DNA extraction methods, were evaluated as far as their degradation levels were concerned. For this purpose the primer pair IVR1-F/IVR1 (Ehlers et al., 1997) was used and able to amplifie 226 bp fragments from invertase gene in maize DNA. All DNA from tested samples were given positive vit IVR1-F/IVR1 (Figure 3). The results indicated that the extracted DNA has high quality and free from any inhibitor beacuse these primer pairs served as a control for the amplification of the isolated DNA and PCR procedure. The primer pair Cry03/Cry04 able to amplify 211 bp from Transition site from the CCDPK-promoter into the amino terminal sequence of synthetic Cry1A(b) gene in Bt 176 maize. (Anonymus (2002).

Specific detection and identification of genetically modified maize DNA:

All the positive samples results from GMOScreen 35S/NOS test kit were positive for 35S promotor only, these may be one from these maize varieties MaximizerTM Bt176, LibertylinkTM corn, B16 corn, Bt-XtraTM corn, Pioneer-MS corn, Herculex TM ITC1507 Maiz and Herculex TM Rootworm DAS 59122-7. The positive samples were identified by using of PCR, the

primer pair IVR1-F/IVR1 (Ehlers et al., 1997) was used and able to amplifie 226 bp fragments from invertase gene in maize DNA as a control. The primer pair Cry03 /Cry04 able to amplifiy 211 bp from transition site from the CCDPK-promoter into the amino terminal sequence of synthetic Cry1A(b) gene in Bt 176 maize. (Anonymus (2002).

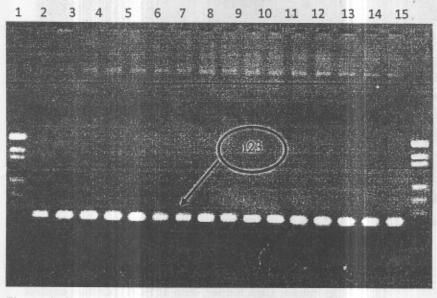


Figure 2. Example for detection of the 35S Promoter in maize samples collected from Egyptain market. Analysis was performed and is documented as described in legend to figure 1 except that the GMOScreen 35S/NOS test kit for qualitative detection of GMO varieties in food, feed test kit was used for PCR-analysis. Lane: 1-18: lane 2: PCR control with DNA positive provide with kit; lanes 3+4+5+6: DNA from USA maize kernels; lane 7+8+9: DNA from Egyptian maize; lane 10+11: DNA from corn flour; lane 12+13 DNA from maize snacks; lines14: DNA from cannend (sweet maize); line 15: DNA from frozen maize; line 16: DNA from corn flacks.

The amplicom results from the positive samples raised at 211 bp. Figure (4) indicated the positive results obtained from the investigation maize samples. 17 samples from 50 invesyigation samples were give positive results for maize lines Bt 176. The obtained results indicated that the presence of genetically modified foods in Egyptian food market. Similary results were obtained by Zbigniew et al., (2006) and Milia et al., (2008) who found that the Egyptian food market contained genetically modified food with out any labllens, and found genetically modified Roundup Ready soybean in

processed food that collected from Italian food market and found genetically modified maize and sovbean in animal feedingstuffs in poland.

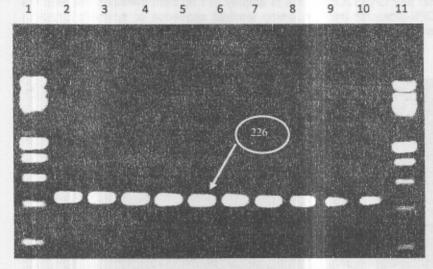


Figure 3. Detection of the of the maize invertase gene in different food samples. The size and location of the expected amplification product is indicated. DNA was extracted from different samples and examined by PCR-analysis using primer pair primer pair ivr1-f/ivr1-r was used for PCR-analysis. Lane 1-12: 100 bp marker DNA ladder, lane 1: PCR positive control DNA from 0.5% genetically modified Bt 176 maiz. Line 3: bread with corn flour toritale.; lanes 4 -11; DNA from different maize samples; Lanes: 4: DNA from maize kernals from USA; lane 5: DNA from maize flour; lane 6: DNA from cannend maize); lane 7: DNA from frozen maize (sweet corn); lane 8: DNA from starch; lane 9: DNA from extruded maize; line 10: DNA from Popcorn; line 11: corn flacks.

Sensitivity of detection:

By using specific primers (see Table 2). For the identification of Bt176 maize using the respective primer pairs DNA 0.5 % GMO material (CRM) was detectable using the present PCR set up as presented in Figure (4) (lane 2).

In conclusion ,the extraction methods that used in extraction of genomic DNA have a great influence in both quality and quantity of DNA extraction. The selection and application of specific DNA extraction method in the a practiculer labouratory must be taken in account in the requirerments of experminetal work flow with respect to samples type and throughput, as well as the cost and time. The choice for the method should be by understanding the practical aspects of implementing a particular methods downstream

aplication. The extraction and purification of DNA can decrease the amount of inhibitory substances to avoid falsh negative especially in quality control of genetically modified foods, feeds and crops. High pure GMO sample preparation Kit Roche diagnostics, which showed the highest amplicon length obtained with different investigation samples DNA. The results clearly demonstrate the incidence of genetically modified maize on the Egyptian food market in raw materials and processed products. The Egyptain maize varieties were found positive for Bt 176 maize this results may be due to genes flow from GM maize to another varieties. Further study will be undertaken to detect unauthorised GMOs for food use in Egypt, and determine the GMO % in the samples.

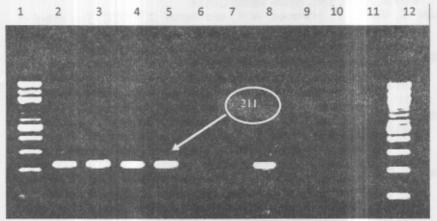


Figure 4. Detection of the of the maize invertase gene in different food samples. The size and location of the expected amplification product is indicated. DNA was extracted from different samples and examined by PCR-analysis using primer pair primer pair CRY03/CRY04 was used for PCR-analysis. Lane 1-12: 100 bp marker DNA ladder, lane 2: PCR positive control DNA from 0.5% genetically modified Bt 176 maiz; lanes 3 -11; DNA from different maize samples. Line 3: cannend maize; Lanes: 4: DNA from maize kernals from USA; lane 5: DNA from maize flour; lane 6: DNA from bread with corn flour (toritale); lane 7: DNA from frozen maize (sweet corn); lane 8: DNA from corn flacks; lane 9: DNA from extruded maize; line 10: DNA from Popcorn; line 11: starch.

REFERENCES

- Ahmed, F. E.(2002). Detection of genetically modified organisms in food. Trends in Biotech. 20: 215-223.
- Anklam, E., Gadani, F., Heinze, P., Pijenburg, H.; Eede, V. G. (2002).

 Analytical methods for detection and determination of genetically modified organisms in agriculture crops and plant derived food products. Eur Food Res Technol, 214, 3-26.
- Anonymus (2002). No L -15.05.1, Amtlich Sammlung von Untersuchungsverfahren nach § 35 LMBG. Untersuchung von Lebensmitteln: Nachweis gentechnischer Veränderungen in Mais (Zea mays L.) mit Hilfe der PCR (Polymerase Chain Reaction) und Restriktionsanalyse oder Hybridisierung des PCR-Produktes Loose leaf edition, Berlin, Köln, Beuth Verlag GmbH.
- Bauer, T., Weller, P., Hammes, W.P., and Hertel. C. (2003). The effect of processing prameters on DNA degridation in foods. European Food Research Technology. 217; 338-343
- Chapela, M.J., Sotelo, C., Perez-Martin, I-R., Pardo, A.M, Perez-Villareal, B., Gilardi, P.Riese, J. (2007) Comparison of DNA extraction methods from muscle of canned tuna for species identification. Food Control,18: 1211-1215.
- Ehlers, B., Strauh, E., Goltz, M., Kubsch, H., Wagner, H., Maidhof, J., Bendiek.B. Appel und Buhk, H.J. (1997). Nachweis gentechnischer Veränderungen in Mais mittels PCR. Bundesgesundheit-blatt 4: 118-121
- Elke, A., Ferruccio, G., Petra, H., Hans, P., and Guy, V. D. E. (2002). Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food product. Eur. Food Res. Technol. 214: 3-26.
- Elsanhoty, R., Boegl, K. -W., Zagon, J., Flachowsky G. (2005).

 Development of a construct-specific, qualitative detection method for genetically modified potato *spunta* in raw potato and potato-derived products. Proceedings of researches delivered at 9 th Egyptian conferences of home Economic. 19-20 September 2005
- Elsanhoty, R., Shahwan, T., and Ramadan, M. F. (2006). Application of Artificial neural networks to develop a classification model between genetically modified maize (Bt-176) and conventional maize by applying lipid analysis data. Journal of Food Composition and Analysis. 19: 628–636.
- Gachet, E., Martin, G. G., Vigneau, F., and Mayer, G. (1999). Detection of genetically modified organisms (GMOs) by PCR: a brief review of methodologies available. Trends in Food Science & Technology. 9: 380-388.
- Hupfer, C., Hotzel, H., Sachse, K. (1998). Detection of the genetic modification in heat-treated products of Bt maize by polymerase chain reaction. Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 206,:203-207.

- Milia, M., Vodret, B., Serratrice, G., Soro, B., Mancuso, R-M. (2008). Three different extraction methods for detecting Roundup Ready Soybean in processed food from the Italian market. Inter. J. Integra. Biol., 3: 2, 133-130.
- Olexova, L., DovicovicovJ, L., & Kuchta, T. (2004). Comparison of three types of methods for the isolation of DNA from Xours, biscuits and instant paps. *European Food Research Technology*, 218, 390–393.
- Peano, C., Somson, C.M., and Palmieri, L. (2004). Qualitative and quantitative evalution of the Genomeic DNA extracted from GMO and Non-GMO FoodStuffs with four different extraction methods. J. Agric. Food Chem. 52, 6962-6968.
- Sambrock, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press p. 6.15.
- Simith, D.S., Philip, M.W., Solke H.D. (2005). Comparison of several methods for the extraction of DNA from potatoes and potato-derived products. Journal of agricultural and food chemistry, 53:, 9848-9859
- Sisea C. R., . D. Pamfil1. (2007). Comparison of DNA extraction methods on DNA for GMO analysis of food products.Bulletin USAMV-CN. 63 64
- Terry, C.F., Harris N., and Parkes, H.,C.(2002). "Detection of Genetically Modified Crops and their Derivatives. Critical steps in sample preparation and extraction." (2002) Journal of AOAC int., 85: 3, 768-774.
- Toyota, A., Akiyama, H., Sugimra, M., Watanbe, Hiroyuki, T., Hisayuki, K., HINO K-A., ESAKA, M., Maitani, T. (2006). Quantification of genetically modified soybeans using a combination of a capillary-type Real-Time PCR system and a plasmid Reference Standard. Biosci. Biotechnol. Biochem., Vol. 70, 821-827.
- Vodret, B. Milia, M., Orani, M., Serratrice, G., and Mancuso, M.R. (2007).

 Detection of Genetically Modified Organisms in Food: Comparison
 Among Three Different DNA Extraction Methods. Veterinary Research
 Communications, 31: 1, 385-388.
- Yamaguchi, H., Sasaki, K., Umetsu, H., and Kamada, H. (2003). Two detection methods of genetically modified maize and the state of its import into Japan. Food control, 14: 201-206.
- Yohimitsu, M., Hori, S. (2003). Comparison of DNA extraction methods from potato snacks and detection of genetically modified potato snacks. Jpn. J. Food Chem., 10: 3, 23-27.
- Zbigniew, S., Malgorzata, W., Krzyztof, K. (2006).Occurrence of genetically modified maize and soybean in animal feeding stuffs. Bull Vet Inst Pulawy 51, 567-570

الكشف عن الأغذية المعدلة وراثيا: مقارنة طرق استخلاص ال (د ن أ) للسذرة الخام المعدلة وراثيا ومشتقاتها.

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هذا العمل تم أجر اؤة للمقارنة بين ست طرق لاستخلاص ال (دن أ) من النزة الخام ومشتقاته. هذا بالإضافة إلى اختيار الطريقة التي تعطى ناتج تركيز عـــالـي مـــن ال (د ن أ) وذات جودة عالية للكشف عن التغيرات الوراثية في العينات الغذائية (·· عينة) المحتوية على الذرة التي تم تجميعها من السوق المصرية. وهذه الطرق استخدمت وقيمت لاستخلاص ال (دن ١) مسن للعينات الغير معاملة تصنيعيا مثل حبوب الذرة والمعاملة ميكانيكيا مثل دقيق الذرة والذرة السكرية المعبأة في علب (المعلبة) والذرة الحلوى المعبأة المجمدة. والعينات المعاملة حراريا وميكانيكيا مثل نشًا الذرةَ فيشار الذرة والكورن فليكس وقراميش الذرة والخيز المضاف إليــه دقيــق الـــذرة. تـــم أستخلاص ال (د ن أ) ال من العينات المختلفة و قياس جودته وكميتة مقارنة بعينــة ذرة قياســية معروف نسبة المعدل وراثيا بها وهمم كمادة قياسمية. واختبسرت العينسات بطريقسة الجيسل اليكتروفوريسيس. واختبرت العينات للكشف عن العوامل الوراثية الموجودة في معظم المحاصيل المهندسة وراثيا باستخدام الكيتس المنتج بواسطة شركة جين إسكان للتعــرف علــــي ال (نـــوس بروموتر) و ال (سيرتي فيف اس ترميناتور). والعينات التي أعطت نتيجة موجبة لوجودهمــــا تــــم التعرف على الصنف الذي تحتوي عليهما باستخدام الطرق القياسية التي أقرها الاتحاد الأوربسي. واظهرت النتائج أن كل الطرق المستخدمة أعطت نتيجة جيده عند استخدامها مع العينات الخام الغير معاملة تصنيعيا. ولكن أعطى الكيتس المنتج بواسطة شركة روش المسمى (بالهاي بيور) اعلى درجة عالية من الجودة النوعية والكمية مقارنة بباقى الطرق وكان من الملاحظ انسه يقل تركير ال دن ا في العينات المعاملة تصنيعيا. وإن الكيتس المنتج بواسطة شسركة روش المسمى باسم (الهاي بيور د ن أ) هو الأفضل عن باقى الطرق في جميع العينسات ســواء كانـــت الـخـــأم لوالمصنعة. ومن ناحية أخري أظهرت نتاتج الدراسة المسحية أن ١٧ من العينات المحتوية علــــى⁻ الذرة موجبة لوجود ال (سيرتي فيف اس بروموتر). وبالتعرف عليها وجد ٣٤% أنها معدلة وراثيًا لصنف الذرة ال (ب تي ١٧٦). من هذه النتائج يتضح أن هناك أغذية معدلـــة وراثيـــا موجـــودة بالأسواق المصرية بدون أي أغلفة توضح للمستهلك أنها معدلة ور اثيا.