qPCR-based Detection of Genetically Modified Processed Foods in India

INVESTIGATORS

Dr. Vinod Vijayan

Dr. Rajarshi Banerjee

July 2018

CENTRE FOR SCIENCE AND ENVIRONMENT (CSE)
41, TUGHLAKABAD INSTITUTIONAL AREA, NEW DELHI–110062
TEL:91-11-29956110/5124/6394/6399
FAX:91-11-29955879 EMAIL: cse@cseindia.org
WEBSITE: www.cseindia.org

POLLUTION MONITORING LABORATORY,
ANIL AGARWAL ENVIRONMENT TRAINING INSTITUTE (AAETI),
(A unit of Centre for Science & Environment, New Delhi)
NO.2151/2036 & 2037/2083, NIMLI, TIJARA, ALWAR, RAJASTHAN-301019

Contents

qPCR-based Detection of Genetically Modified Processed Foods in India

1. INTRODUCTION

In order to regulate the availability of genetically modified (GM) foods in a country, approval process and enforcement systems require detection of foods prepared with ingredients from genetically modified organisms (GMOs) using a reliable and precise DNA-based (deoxyribonucleic acid) method. As varieties of GM crops are increasing, it is important to use appropriate qualitative methods for screening GM ingredients in food products (Maryam Rabiei, 2013). The basis for such a qualitative GM screening procedure involves the detection of genetic sequences such as promoters and transcription terminators. GM crops contain promoter sequences like 35S promoter of cauliflower mosaic virus (CaMV) and FMV promoter of figwort mosaic virus, and terminator sequence like 3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*. It is possible to detect such sequences by real time quantitative Polymerase Chain Reaction (qPCR) method (Reinhard Zeitler, 2002). More than 95 per cent of the presently available GM crops are positive for a combination of a these promoter and terminator sequences (Markus Fandke, 2002).

Country-level legislation should ensure a safe approach to GMOs, GM food, and GM feed, which includes food and environmental safety. It should cover all aspects such as cultivation, manufacturing, import and sale and ensure appropriate authorization, traceability and labelling. Qualitative detection is the first critical and effective step in GMO analysis, as only positive samples are subjected to further identification and quantitative analysis.

The apex food regulator in India − food safety and standards authority of India (FSSAI) has so far not approved any GM food product to be manufactured, distributed, sold or imported in the country. The objective of this study was to check the availability of processed GM foods in the Indian market through the use of qPCR screening procedure.

2. MATERIALS AND METHODS

2.1. Sample collection of food commodities – oils, packaged foods, infant foods and protein supplements from retail shops

Processed foods which could possibly have commonly marketed GM ingredients were identified. These include food products that were made from or using soya, corn, cotton and rapeseed (canola) ingredients as GM variants of these crops are widely cultivated in many parts of the world. The samples were randomly collected from retail outlets in Delhi NCR, Gujarat and Punjab and included those domestically-manufactured or imported into India. Some food products declared the use of GM ingredients on their labels (*see Table 1: Samples tested for GMO screening; see Annexure 1: Details of food samples tested).*

To corroborate study results, cotton leaf (collected from Nimli, Tijara in Rajasthan) and commercially available cottonseeds (Super-931, Bollgard II, Monsanto) were collected and used as positive controls for testing GM material.

Table 1: Samples tested for GMO screening

Food product/ category	Brand/company	Relevant ingredients	Labelling with regard to GM	Country of origin
Oils: 16				
Canola	Wagga Wagga (Diabetes care)	Edible vegetable oil. Imported refined rapeseed oil (canola oil) low erucic acid	Made from Non-GMO Canola seeds	Australia
Canola	Wagga Wagga (Heart care)	Imported refined rapeseed oil low erucic acid (80% by wt), imported refined olive oil (20% by wt).Seeds from Australia.	-	India*
Canola	Borges	Edible refined rapeseed low erucic acid oil	-	Spain
Canola	Jivo	Imported refined rapeseed oil low erucic acid oil	-	Canada
Canola	Candrop	Imported rapeseed low erucic acid oil (canola oil). Product of Canada	GMO free	Canada
Canola	Farell	Edible vegetable oil. Imported refined rapeseed oil low erucic acid oil	-	UAE
Canola	Hudson	Imported refined canola oil. Edible grade oil. Extracted from Canadian oilseeds	-	UAE
Soya bean	Fresh & Pure	Refined soya bean oil	-	India
Soya bean	Fortune Soya Health	Refined soya bean oil	-	India
Soya bean	Nature Fresh Acti Lite	Soya bean oil	-	India
Corn rice bran	Saffola Tasty	60 % corn oil, 40 % rice bran oil	-	India
Cottonseed (kapasia tel)	Tirupati	Refined cottonseed oil	-	India
Cottonseed (kapasia tel)	Ankur	Refined cottonseed oil	-	India
Cottonseed	Ginni	Refined cottonseed oil	-	India
Cottonseed	Vimal	Refined cottonseed oil	-	India
Crude cottonseed	Unknown	Crude cottonseed	-	India
Packaged foods: 39				
Soya vadi	Golden Harvest	Not mentioned	-	India
Mini soya chunks (mini soya vadi)	Fortune	Soya flour	-	India
Soya chunks	Nutrela	100% defatted soya	-	India
Soya chunks	Ektaa	Not mentioned	-	India
Dark soy sauce	Pantai (norasingh)	Soya bean extract (52%), soya bean	-	Thailand
Dark soy sauce	Ong's	Soya bean extract (38%), soya beans	-	Singapore
Soy sauce classic	American Garden	Hydrolysed soy protein, high fructose corn syrup	-	USA
Corn flakes + (with real honey)	Bagrrys	Corn grits (85%), invert syrup	-	India
Corn flakes	Kellogg's	Corn grits, invert syrup	-	India
Sweet corn chicken (instant everyday soup)	Keya	Corn	-	India
Sweet corn soup	Ching's Secret	Corn starch, sweet corn	-	India
Sweet corn chicken soup	Knorr Classic	Maize starch, corn (5.8%)	-	India
Frozen sweet corn	Safal	Individually quick frozen sweet corn kernels	-	India
Sweet corn kernel	Veg affaire	Individually quick frozen sweet corn kernels	-	India
Whole kernel corn	Del Monte	Corn	-	India
Sweet whole kernels corn	PromPlus	Sweet kernel corn	Non-GMO	Thailand
Corn (original naturally sweet)	Green Giant	Sweet corn	-	France
Popcorn (classic salted)	ACT II	Popping corn, refined edible palmoline oil	-	India
Sriracha lime cheese popcorn	PVR	Corn (62%), edible vegetable oil	Non-GMO corn	India
American Sweet Corn	Yummiez	Sweet corns	-	India
Pringles potato crisps (original)	Kellogg's	Corn flour	-	Malaysia
Potato crisps	Lays (Stax Original)	Cottonseed oil, corn oil, soy lecithin, contains a soy ingredient	Partially produced with	Mexico

Food product/ category	Brand/company	Relevant ingredients	Labelling with regard to GM	Country of origin
			Genetic Engineering	
Froot Loops (sweetened multigrain cereal)	Kellogg's	Corn flour blend, whole grain yellow corn flour, degerminated yellow corn flour, soybean and /or cottonseed oil	-	USA
Tortilla chips (cool ranch flavoured)	Doritos	Corn, corn oil, canola oil, corn starch, corn syrup solids, maltodextrin made from corn	-	USA
Crunchy peanut butter	American Kitchen	Hydrogenated vegetable oil (cottonseed and/or rapeseed oil)	-	USA
Popcorn Hot N' Spicy	American Garden	Partially hydrogenated soybean oil, popcorn, contains milk and soy	-	USA
Butter and Garlic Croutons made from Texas Toast Bread	Mrs. Cubbison's	Canola oil, high fructose corn syrup	Partially produced with Genetic Engineering	USA
Multigrain Cheerios (5 whole grains)	Nestle	Wholegrain corn flour (2%), partially inverted brown sugar syrup	-	UK
Corn puffs (naturally fruit flavoured sweetened)	Trix	Whole grain corn, corn meal, corn syrup, canola oil	Produced with Genetic Engineering	USA
Aloo bhujia	Haldirams	Cottonseed oil, corn oil	-	India
Pop tarts toaster pastries (frosted blueberry)	Kellogg's	Corn syrup, high fructose corn syrup, soybean oil, corn flour, soy lecithin, corn starch, contains wheat and soy ingredients	-	USA
Silken tofu (extra firm)	Mori-Nu	Soybeans, isolated soy protein	Non-GMO Project Verified	USA
Soya milk (strawberry)	Soyfresh	Soybean extract (85%), corn oil Made From 100% Canadian soybean	-	Malaysia
Panchrattan Namkeen	Haldirams	Cottonseed oil, corn oil	-	India
Crispy corn snacks (original flavour)	Bugles	Degermed yellow corn meal	-	USA
Chicken corn coating (Mingles bucket)	KFC	Corn flour	-	India
Original syrup	Aunt Jemima	Corn syrup, high fructose corn syrup (HFCS)	Produced with Genetic Engineering	USA
Dark corn syrup	Karo	Corn syrup, refiners syrup	Produced with Genetic Engineering	USA
Pancake syrup original	American Garden	High fructose corn syrup (HFCS), corn syrup	-	USA
Infant foods: 8				
Infant formula	Similac IQ+ 1	Soy oil	-	Singapore
Soy infant formula (lactose-free infant milk substitute, soy infant formula)	Similac Isomil (up to 24 months)	Hydrolysed corn starch, soy protein isolate (15.61%), soy oil	-	Netherlands
Follow-up formula for older infants	Enfamil A+ 3 (from 12 to 24 months)	Vegetable oil, low erucic rapeseed oil, corn syrup solids, corn oil	-	Thailand
Hypoallergenic formula	Nutramigen LGG	Corn syrup solids, soybean oil, modified corn starch	-	Netherlands
Lactose and sucrose free infant milk substitute	Dexolac Nusobee Soya (up to 24 months)	Soya oil, soya protein isolate	-	India
Follow-on milk	SMA pro 2	Emulsifier (soy lecithin), rapeseed oil	-	Ireland
Hypoallergenic infant formula	Similac Alimentum (from birth onwards)	Maltodextrin, soy oil, emulsifier modified corn starch	-	USA
Lactose and sucrose free infant milk substitute, type II	Zerolac	Soya protein isolate (16.2%)	-	India
Protein supplements: 2				
Protein supplement for adults	Prosure (delicious banana flavour)	Soya oil, soya polysaccharide, soya lecithin	-	Spain
Soybean-based nutritional beverage mix	Protinex (elaichi flavour, low fat)	Soya protein isolate, corn flour	-	India

*'-' denotes no labelling with regard to GM; * considered imported as seeds are from Australia*

2.2. DNA extraction from test sample

DNA was extracted using DNeasy mericon Food Kit (Qiagen, USA) as per manufacturers' instructions. Briefly, 2 g of food material was ground using a sterile mortar and pestle with liquid nitrogen. 10 ml food lysis buffer and 25 µl Proteinase K solution was added to the homogenized food sample in a 50 ml centrifuge tube and vortexed briefly. Evenly distributed and moistened food sample was incubated at 60°C with constant shaking at 100 rpm for 30 min, followed by cooling to room temperature (15-25°C) on ice to enhance inhibitor precipitation. The tubes were centrifuged for 5 min at 2500 x g (Hanil, South Korea). 1 ml of the clear supernatant was transferred carefully to a microcentrifuge tube containing 500 µl chloroform followed by rigorous vortexing for 15 s. The microcentrifuge tubes were centrifuged at 14,000 x g for 15 min (Hanil, South Korea). The aqueous phase was collected and mixed with 350 µl buffer PB in a fresh 2 ml microcentrifuge tube. The contents were filtered through a QIA quick spin column at 17,900 x g for 1 min followed by addition of 500 µl buffer AW2 and a 1 min spin at 17,900 x g, discarding the flow through and drying the column at 17,900 x g for 1 min. The DNA was eluted using 50 µl buffer EB and spinning the column at 17,900 x g for 1 min. For liquid samples *viz.* oil, soy milk and corn syrup, 300 ml of the test sample was centrifuged at 24,000 x g for 30 min (Elke Anklam, 2002; European Commission, 1999; M Hellebrand, 1998). The pellet was treated as per the protocol cited above to extract the DNA, which was then subjected to electrophoresis on ethidium bromide-containing 1% agarose gel and visualized using gel documentation system (DNS, Israel). See Annexure 3: List of abbreviations of unit symbols used in sections 2.2 and 2.3

2.3. qPCR analysis

qPCR for detecting GM DNA in food samples was done using the SureFood® GMO SCREEN 4plex (35S/NOS/FMV+IAC) detection kit (CONGEN Biotechnologie GmbH) purchased from R-Biopharm AG, Germany as per the manufacturers' protocol. The multiplexing PCR kit allows for the detection of 35S promoter, NOS terminator and FMV promoter. The assay is compatible with instruments such as Quantstudio-5 (Thermo, USA) used in the present study that can detect fluorescence emissions at 522 nm, 553 nm, 610 nm, and 670 nm (FAM, VIC, ROX, and Cy5) simultaneously. The limit of detection (LoD) of the assay is ≤5 DNA copies. The kit is compliant to the German Food Law § 64 for detecting GM markers.

qPCR method was also validated using positive control (DNA from i. cotton leaf sourced from Nimli, Tijara in Rajasthan, ii. Super-931, Bollgard II, Monsanto Bt seed and iii. internal amplification control supplied by the kit manufacturer) and negative control (sterile molecular grade water). The plots for qPCR amplification are shown in Annexure 2. Thermal cycle was programmed for 5 min at 95°C for initial denaturation, followed by 45 cycles of 15 s at 95°C for amplification and 30 s at 60°C for final annealing/extension (s*ee Table 2: Components of qPCR mix).* Samples were tested in triplicates. The results were evaluated using QS v.1.3 program.

Table 2: Components of qPCR mix

Component	Amount per reaction
Reaction mix	19.9 µl
Taq Polymerase (5 U/µl)	0.1 µl
Total volume	20 µl
Sample DNA	5 µl
Total Reaction volume	**25 µl**

3. RESULTS

A total of 65 food product samples (listed in Table 1) were tested for the presence of GM markers. DNA was isolated from each food sample and subjected to qPCR analysis to identify the presence of three GM marker genes (35S promoter, NOS terminator and FMV promoter) by a standardized protocol (listed in materials and methods).

Obtaining good quality DNA is key to reliable qPCR results, hence before testing the DNA samples for GM marker genes, the concentration, purity and integrity of the DNA isolated from control samples was confirmed by visualizing the DNA on a 1 % ethidium bromide-containing agarose gel (Figure 1).

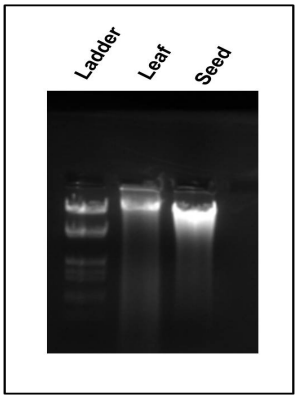


Figure 1: Good quality DNA isolated from positive control samples (cotton leaf and cottonseed). A DNA ladder is provided for reference.

Overall, 32 per cent (21/65) food product samples tested were GM-positive (*see Table 3A: List of GM positive food products and Ct values; see Table 3B: Ct values of positive control samples; see Annexure 2: qPCR amplification plots for GM markers in food and control samples)*. Eighty percent (16/21) of these were imported food samples. About 46 per cent (16/35) imported food products were positive. These were imported from Canada, the Netherlands, Thailand, UAE, and the US. About 17 per cent (5/30) samples manufactured in India were positive. GM positive imported food products were based on or used soya, corn and rapeseed. All positive samples manufactured in India were based on cottonseed i.e. cottonseed oil. This includes samples of four packaged cottonseed oil and one crude cottonseed oil. Fifty six per cent (9/16) of oil samples, 25 per cent (10/39) of packaged food samples and 25 per cent (2/8) infant food samples were found GM-positive. Two protein supplement samples tested were GM-negative.

Out of the 20 GM-positive packaged samples (excluding crude cottonseed oil), 65 per cent (13/20) did not mention use of GM ingredients on their labels. These include canola oil brands – 'Farell', 'Hudson', and 'Jivo' and cottonseed oil brands – 'Ankur', 'Ginni', 'Tirupati' and 'Vimal'; packaged foods like 'Pancake syrup original', 'Popcorn Hot N' Spicy', 'Froot Loops' and 'Crispy corn snacks'; Infant foods like 'Similac Isomil' and 'Similac Alimentum'. Three samples which suggested no use of GM ingredients on their labels but were found GM-positive are 'Candrop' canola oil, 'Mori-nu Silken tofu' and 'PromPlus sweet whole kernel corn'. Four samples which labelled about use of genetic engineering technology were 'Butter and Garlic Croutons', 'Corn puffs', 'Original syrup' and 'Dark corn syrup'.

Samples of soyabean oil, blended corn rice bran, packaged foods and infant foods manufactured in India were found GM negative.

Table 3A: List of GM positive food products and Ct values

Food product/ category	Brand/ company	Target GM marker*	Mean Ct value	Food product/ category	Brand/ company	Target GM marker*	Mean Ct value
Oils				**Packaged foods**			
Canola	Hudson	35S P	26.7	Sweet whole kernels corn	PromPlus	35S P	26.4
		FMV P	29.5			FMV P	29.3
		T NOS	26.5			T NOS	26.0
Canola	Jivo	35S P	28.1	Original syrup	Aunt Jemima	35S P	29.6
		FMV P	29.6			FMV P	31.3
		T NOS	26.3			T NOS	29.3
Cottonseed	Vimal	35S P	25.5	Dark corn syrup	Karo	35S P	27.3
		FMV P	29.6			FMV P	31.3
		T NOS	26.0			T NOS	29.4
Cottonseed (kapasia tel)	Tirupati	35S P	27.6	Pancake syrup original	American Garden	35S P	30.6
		FMV P	29.7			FMV P	31.1
		T NOS	27.1			T NOS	29.2
Cottonseed (kapasia tel)	Ankur	35S P	27.2	Froot Loops (sweetened multigrain cereal)	Kellogg's	35S P	20.7
		FMV P	29.5			FMV P	26.2
		T NOS	26.6			T NOS	22.4
Crude cottonseed	Unknown	35S P	17.2	Crispy corn snacks (original flavour)	Bugles	35S P	26.2
		FMV P	26.8			FMV P	28.7
		T NOS	19.0			T NOS	26.1
Canola	Farrell	35S P	26.5	Popcorn Hot N' Spicy	American Garden	35S P	28.6
		FMV P	29.8			FMV P	32.1
		T NOS	26.8			T NOS	27.0
Canola	Candrop	35S P	24.0	Butter and Garlic Croutons made from Texas Toast Bread	Mrs. Cubbison's	35S P	28.0
		FMV P	29.8			FMV P	29.6
		T NOS	25.8			T NOS	26.7
Cottonseed	Ginni	35S P	22.4	Corn puffs (naturally fruit flavoured sweetened)	Trix	35S P	25.0
		FMV P	29.3			FMV P	29.5
		T NOS	23.4			T NOS	26.8
				Silken Tofu (extra firm)	Mori-Nu	35S P	25.9
						FMV P	29.6
						T NOS	26.4
Infant foods							
Soy infant formula (lactose-free infant milk substitute, soy infant formula)	Similac Isomil (up to 24 months)	35S P	29.2	Hypoallergenic infant formula	Similac Alimentum (from birth onwards)	35S P	30.7
		FMV P	29.8			FMV P	31.2
		T NOS	27.4			T NOS	29.1

Table 3B: Ct values of positive control samples

Sample	Sourced from	Target GM gene*	Mean Ct value	Sample	Sourced from	Target GM gene*	Mean Ct value
Cotton leaf	Nimli, Tijara, Rajasthan	35S P	28.5	Cotton seed	Super-931, Bollgard II, Monsanto	35S P	24.9
		FMV P	29.5			FMV P	29.4
		T NOS	26.9			T NOS	25.6

*Note: *35S P = 35S promoter, FMV P= FMV promoter, T NOS= NOS terminator*

4. CONCLUSION

DNA-based qPCR method is a reliable method for screening GM markers in processed food samples, which helps to identify GM food. In the past, studies have been conducted using this method for screening GM in food collected from consumer markets. Examples of such studies include those from Romania (Elena Rosculete, 2018), Oman (Nabila Al-Sadqi, 2016), Egypt (El Sanhoty, 2002), Turkey (Merve Mandaci, 2014), Tunisia (Maher Chaouachi, 2013), and Jordan (Al-Hmoud N, 2010).

The present study shows that it is possible to screen GM in processed foods. It identifies GM foods (including imported and domestically-manufactured) that are commonly available in the Indian market.

The study results call for screening of all foods made from or likely containing GM ingredients in the country. The FSSAI should set up systems and enforce GM screening which would help in regulating approval, traceability and labelling of GM foods.

5. REFERENCES

Maryam Rabiei, Mehrangiz Mehdizadeh, Hossein Rastegar, Hossein Vahidi, and Mahmoud Alebouyeha (2013). Detection of Genetically Modified Maize in Processed Foods Sold Commercially in Iran by Qualitative PCR. Iranian Journal of Pharmaceutical Research*12* (1): 25-30.

Reinhard Zeitler, Klaus Pietsch, Hans-Ulrich Waiblinger (2002). Validationof real-time PCR methods for the quantification of transgeniccontaminations in rapeseed. European Food Research and Technology*214* (4):346-351.

Markus Fandke, Astrid Schneider, Cordt Grönewald, and Kornelia Berghof-Jäger (2002). Real-Time PCR Screening of Genetically Modified Organisms with the Light Cycler Instrument. Biochemica*2*: 14-16.

Elke Anklam, F. G. (2002). Analytical methods for detection and determination of genetically modified organisms in agricultural crops and plant-derived food products. European Food Research and Technology*, 214* (1), 3–26.

European Commission (1999, June 17). Retrieved July 16, 2018, from Concerning the scientific basis for determining whether food products, derived from genetically modified soya and from genetically modified maize could be included in a list of food products which do not require labelling because they do not contain (detectable) traces of DNA or protein: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out33_en.pdf

M Hellebrand, M. N.-T. (1998). Determination of DNA traces in rapeseed oil. Zeitschrift für Lebensmitteluntersuchung und -Forschung A*, 206* (4), 237–242.

Elena Rosculete, Elena Bonciu, Catalin Aurelian Rosculete, and Elena Teleanu (2018). Detection and Quantification of Genetically Modified Soybean in some food and feed products. A case study on products available on Romanian market. Sustainability*10* (1325): doi-10.3390/su10051325.

Nabila Al-Sadqiand Aliya S. Alansari (2016). Detection of Unlabeled Genetically Modified Soybean in the Omani Market.SQU Journal for Science*21*(1): 1-6.

El Sanhoty, H. Broll, L. Grohmann, B. Linke, A. Spiegelberg, K.-W. Bogl and J. Zagon (2002). Genetically modified maize and soybean on the Egyptian food market. FOOD/NAHRUNG*46*: 360-363.

Merve Mandaci, Özgür Çakir, Neslihan Turgut-Kara, Sinan Meriçand Şule Ari (2014) Detection of genetically modified organisms in soy products sold in Turkish market.Food Science and Technology*34* (4): 717-722.

Maher Chaouachi, Nesrine Nabi, Ahmed Ben Hafsa, Mohamed Salem Zellama, Fethia Skhiri, and Khaled Saïd (2013). Monitoring of Genetically Modified Food and Feed in the Tunisian Market Using Qualitative and Quantitative Real-time PCR. Food Science and Biotechnology *22*(4): 1-10.

Al-Hmoud N, Al-Rousan H, Hayek B O, and Ibrahim M A, (2010). Detection of genetically modified maize and soybean food products in the Jordanian market. Biotechnology*9* (4): 499–505.

Annexure 1: Details of food samples tested

Food product/ category	Brand/ company	Manufacturer/distributor/importer	Manufacturing /import date	Expiration date	Batch number	Country of origin	Relevant ingredients
Oils							
Canola	Wagga Wagga (Diabetes care)	Manufactured and packed by Riverina Oils & Bio Energy Pty Ltd, Deepdene, Australia Imported and marketed by Agro global Resources (P) Ltd, Noida, Uttar Pradesh. Packed by Mann Feeds (P) Ltd, Faridabad, Haryana	15 September 2017	12 months	1725812 26**CC	Australia	Edible vegetable oil. Imported refined rapeseed oil (canola oil) low erucic acid
Canola	Wagga Wagga (Heart care)	Manufactured and packed by Mann Feeds (P) Ltd, Faridabad, Haryana Marketed by Agro global Resources (P) Ltd, Noida, Uttar Pradesh	7 September 2017	12 months	1725021 86B*CO/ 03/BVO/ 2017-I III 75.5	India*	Imported refined rapeseed oil low erucic acid (80% by wt), imported refined olive oil (20% by wt). Seeds from Australia.
Canola	Borges	Manufactured by Borges Agricultural and Industrial Edible Oils, Spain. Imported and distributed by Borges India (P) Ltd, New Delhi	May 2017	36 months	173320	Spain	Edible refined rapeseed low erucic acid oil
Canola	Jivo	Imported, packed and marketed by Jivo Wellness Pvt. Ltd, Delhi	December 2017	November 2019	145365.3 1	Canada	Imported refined rapeseed oil low erucic acid oil
Canola	Candrop	Imported, packed and marketed by Century Edible Cooking Oils Pvt. Ltd, Punjab	September 2017	August 2019	A255	Canada	Imported rapeseed low erucic acid oil (canola oil). Product of Canada
Canola	Farell	Manufactured by Al Ghurair Foods LCC, UAE Imported by Jindal Retail (India) Pvt. Ltd, Delhi	2 July 2016	1 July 2018	SN:0907 16	UAE	Edible vegetable oil. Imported refined rapeseed oil low erucic acid oil
Canola	Hudson	Packed for and marketed by Dalmia Continental Pvt. Ltd, New Delhi.	29 December 2017	24months	C52	UAE	Imported refined canola oil, edible grade oil. Extracted from Canadian oilseeds.
Soya bean	Fresh & Pure	Manufactured and packed by Cian Agro Industries & Infra Ltd, Maharashtra. Marketed by Future Consumer Ltd, Mumbai	27 September 2017	9 months	C/FP/SB 0084	India	Refined soya bean oil
Soya bean	Fortune Soya Health	Manufactured by Adani Wilmar Ltd, Ahmedabad, Gujarat	10 October 17	9 months	(AE)SB 08F103.9	India	Refined soya bean oil
Soya bean	Nature Fresh Acti Lite	Manufactured and marketed by Cargill India Pvt. Ltd, Gujarat and Delhi	August 2017	9 months	011163H 07E	India	Soya bean oil
Corn rice bran	Saffola Tasty	Manufactured and marketed by Marico Limited, Mumbai	2 October 17	6 months	JN-238-T	India	60 % corn oil, 40 % rice bran oil
Cottonseed (Kapasia tel)	Tirupati	Marketed by N.K. Proteins Pvt. Ltd, Ahmedabad	9 April 2018	6 months	126M5	India	Refined cottonseed oil
Cottonseed (Kapasia tel)	Ankur	Manufactured and packed by Ambar Protein Industries Ltd, Ahmedabad	9 April 2018	9 months	CH01	India	Refined cottonseed oil
Cottonseed	Ginni	Packed by Bunge India Pvt. Ltd, Punjab	5 January 2018	9 months	BR0118	India	Refined cottonseed oil
Crude cottonseed	Unknown	Unknown, Gujarat	NA	NA	NA	India	Crude cottonseed
Cottonseed	Vimal	Manufactured by Vimal Oil & Foods Ltd, Ahmedabad	23 March 2018	9 months	23	India	Refined cottonseed oil
Packaged foods							
Soya vadi	Golden Harvest	Manufactured and packed by Future Consumer Limited, Samaypur, Delhi	28 September 2017	6 months	SMY171 9	India	Not mentioned
Mini soya chunks (mini soya vadi)	Fortune	For processing and packing unit: Adani Wilmar Ltd, Vidisha, MP Marketed by Adani Wilmar, Gujarat	14 September 2017	12 months	(VA)CK8 9F09	India	Soya flour
Soya chunks	Nutrela	Manufactured and packed by Ruchi Soya Industries Limited, Indore, MP	October 2017	12 months	TC 12 D	India	100% defatted soya
Soya chunks	Ektaa	Manufactured and packed by Future Consumer Limited, MP Marketed by Future Consumer Limited, Mumbai	5 September 2017	12 months	NIM-3447	India	Not mentioned
Dark soy sauce	Pantai	Manufactured by Pantainorasingh Manufacturer Co. Ltd, Thailand	2 February	2 February	TOAB70	Thailand	Soya bean extract (52%),

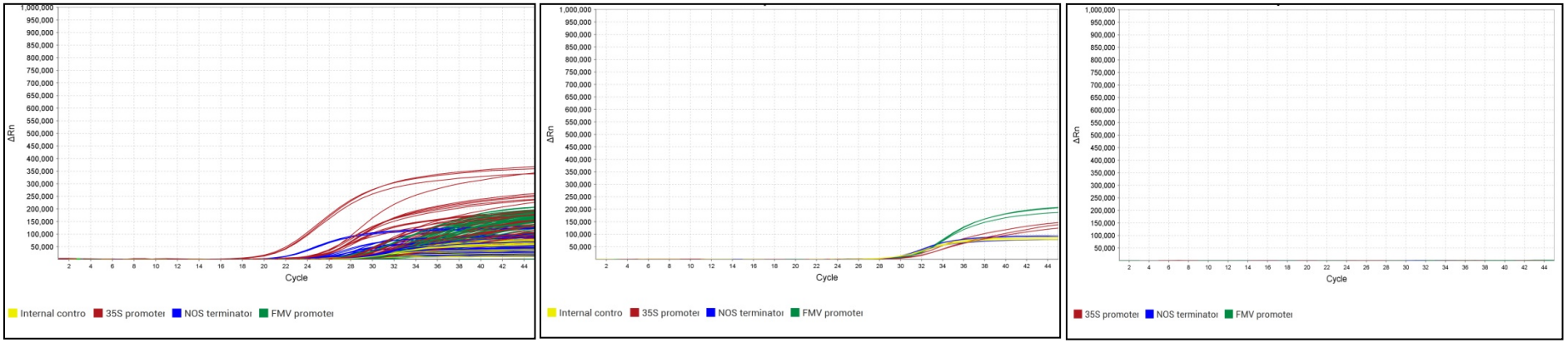
Food product/ category	Brand/ company	Manufacturer/distributor/importer	Manufacturing /import date	Expiration date	Batch number	Country of origin	Relevant ingredients
	(norasingh)		2017	2019	20		soya bean
Dark soy sauce	Ong's	Manufactured by Bachul Food Industries (PTE) Ltd, Singapore Imported in India by Suresh Kumar & Co. (Imtex) Pvt. Ltd, Delhi	3 April 2017	1 April 2020	L17093E 1	Singapore	Soya bean extract (38%), soya beans
Soy sauce classic	American Garden	Product of American Garden, USA Imported and marketed by Bajoria Foods Pvt. Ltd, Mumbai	April 2017	April 2019	E0117AA C	USA	Hydrolysed soy protein, high fructose corn syrup
Corn flakes + (with real honey)	Bagrrys	Manufactured by Bagrrys India Ltd, Baddi, HP Marketed by Barrgys India Ltd, Delhi	17 November 2017	9 months	CFRH32 1	India	Corn grits (85%), invert syrup
Corn flakes	Kellogg's	Manufactured by Kellogg India Pvt. Ltd, Maharashtra	12 November 2017	9 months	T2 C121117	India	Corn grits, invert syrup
Sweet corn chicken (instant everyday soup)	Keya	Manufactured by Keya Foods International Pvt. Ltd, Kerala	2 December 2017	12 months	U700008 A	India	Corn
Sweet corn soup	Ching's Secret	Manufactured by Capital Foods Pvt. Ltd, Gujarat Brand owned and marketed by Capital Foods Pvt. Ltd, Mumbai	18 September201 7	12 months	B17L01K (17:13)	India	Corn starch, sweet corn
Sweet corn chicken soup	Knorr Classic	Manufactured by Hindustan Unilever Limited, Nashik and Avalon Cosmetics Pvt. Ltd, Nashik. Marketed by Hindustan Unilever Ltd (HUL), Mumbai	30 November 2017	12 months	BAS-VI 207	India	Maize starch, corn (5.8%)
Frozen sweet corn	Safal	Packed and marketed by Mother Diary Fruits & Vegetables Pvt. Ltd, Haryana	D/o packaging: November2017	12 months	A17312S C	India	Individually quick frozen sweet corn kernels
Sweet corn kernel	Veg affaire	Packed by Integrated Food Park Pvt. Ltd, Karnataka Marketed by Future Consumer Enterprise Limited, Mumbai	26 August 17	12 months	B6C5260 801	India	Individually quick frozen sweet corn kernels
Whole kernel corn	Del Monte	Manufactured by Field fresh foods Pvt. Ltd, Gurugram, Haryana Marketed by Del Monte (India)	4 October 2017	24 months	HSWO4J 7-5	India	Corn
Sweet whole kernels corn	PromPlus	Produced and packed by Riverquai International Food Industry Co. Ltd. Imported by Gurukirpa Impex, Delhi	20 January 2017, Import: July 2017	36 months	1519	Thailand	Sweet kernel corn
Corn (original naturally sweet)	Green Giant	Manufactured for General Mills International SARL, Switzerland, General Mills, UK	July 2017	July 2021	2137S1	France	Sweet corn
Popcorn (classic salted)	ACT II	Manufactured by Agro Tech Foods Limited, Secunderabad	31 October2017	12 months	3531C30 4P	India	Popping corn, refined edible palmoline oil
Sriracha lime cheese popcorn	PVR	Manufactured and marketed by Zea Maize Pvt. Ltd, Haryana	3 October 2017	2 April 2018	A240310 01	India	Corn (62%), edible vegetable oil
American Sweet Corn	Yummiez	Marketed by Godrej Tyson Foods Ltd, Mumbai	D/o packaging: 7 November 2017	9 months	3110101 P1	India	Sweet corns
Pringles potato crisps (original)	Kellogg's	Manufactured by Super Food Technology, Malaysia Imported and marketed by Kellogg India Pvt. Ltd, Maharashtra	1 January 2018	12 months	8001667 3 20:52f	Malaysia	Corn flour
Potato crisps	Lays (Stax Original)	Manufactured for Frito-Lay Inc., North America Imported and marketed by Newage Gourmet foods, New Delhi	Import: November 2017	18 July 2018	6594131 027	Mexico	Cotton seed oil, corn oil, soy lecithin, contains a soy ingredient
Froot Loops (sweetened multigrain cereal)	Kellogg's	Distributed by Kellog Sales Co., USA Imported and marketed by Newage Gourmet foods, Delhi	Import: March 2018	12 November 2018	3800039 118 Importer: 0260570 1	USA	Corn flour blend, whole grain yellow corn flour, degerminated yellow corn flour, soybean and /or cottonseed oil
Tortilla chips (cool ranch flavoured)	Doritos	Manufactured for PepsiCo Inc. by Frito-Lay Inc., USA Imported and marketed by Newage Gourmet foods, New Delhi	January 2018	31 July 2018	4663011 60	USA	Corn, corn oil, canola oil, corn starch, corn syrup solids, maltodextrin made from corn
Crunchy peanut butter	American Kitchen	Manufactured by American Kitchen, Delware Imported by Sunbeam Mercantile Ventures P Ltd, Cochi	2 March 2017	1 September	Lot No. 0203201	USA	Hydrogenated vegetable oil (cottonseed and/or

Food product/ category	Brand/ company	Manufacturer/distributor/importer	Manufacturing /import date	Expiration date	Batch number	Country of origin	Relevant ingredients
				2018	7		rapeseed oil)
Popcorn Hot N' Spicy	American Garden	Product of American Garden, USA Imported and distributed by Bajoria Foods Pvt. Ltd, Mumbai	25 September 2017	24 months	2687	USA	Partially hydrogenated soybean oil, popcorn, contains milk and soy
Butter and Garlic Croutons made from Texas Toast Bread	Mrs. Cubbison's	Imported and marketed by Newage Gourmet foods, New Delhi	Import: March 2018	30 October 2018	VB63	USA	Canola oil, high fructose corn syrup
Multigrain Cheerios (5 whole grains)	Nestle	Imported and marketed by Newage Gourmet Foods, Delhi	Import: March 2018	June 2018	7199095 3	UK	Wholegrain corn flour (2%), partially inverted brown sugar syrup
Corn puffs (naturally fruit flavoured sweetened)	Trix	Distributed by General Mills Sales Inc., Minneapolis, USA Imported and marketed by Newage Gourmet foods, New Delhi	Import: April 2018	2 September 2018	CEO723 01	USA	Whole grain corn, corn meal, corn syrup, canola oil
Aloo bhujia	Haldirams	Manufactured and marketed by Haldirams Snacks Pvt. Ltd, India	28 April 2018	6 months	H07AB2 80418	India	Cottonseed oil, corn oil
Pop tarts toaster pastries (frosted blueberry)	Kellogg's	Distributed by Kelloggs Sales Co., MI Imported and marketed by Newage Gourmet Foods, New Delhi	Import: January 2018	12 September 2018	CPC3	USA	Corn syrup, high fructose corn syrup, soybean oil, corn flour, soy lecithin, corn starch, contains wheat and soy ingredients
Silken tofu (extra firm)	Mori-Nu	Manufactured by Pacific Nutritional Foods Inc., USA Distributed by Morinaga Nutritional Foods Inc., California Imported by Olive Tree trading Pvt. Ltd, Pune	25 August 2017	25 August 2018	B11:54:0 0	USA	Soybeans, isolated soy protein
Soya milk (strawberry)	Soyfresh	Owner of the rights of manufacture: Ace Canning Corporation Sdn Bhd Manufactured by Okka Ace Lot 39, Keluli1 S.A, Malaysia Imported and marketed by DC Johar & Sons (P) Ltd. Cochin, India	12 January 2018	12 February 2019	12A	Malaysia	Soybean extract (85%), corn oil Made From 100% Canadian soybean
Panchrattan Namkeen	Haldirams	Manufactured and marketed by Haldirams Snacks Pvt. Ltd, India	29 April 2018	5 months	H05PR2 90418 A(27)	India	Cottonseed oil, corn oil
Crispy corn snacks (original flavour)	Bugles	Distributed by General Mills Inc. Minneapolis, USA Imported and marketed by Newage Gourmet Foods, New Delhi	Import: March 2018	25 March 2019	L2341A1 349	USA	Degermed yellow corn meal
Chicken corn coating (Mingles bucket)	KFC	KFC, Gurugram	22 June 2018	22 June 2018	NA	India	Corn flour
Original syrup	Aunt Jemima	Distributed by the Quaker Oats Company, Chicago Imported and marketed by Newage Gourmet Foods, Delhi	Import: March2018	12 December 2019	3000005 970 Importer: 0694320 1	USA	Corn syrup, high fructose corn syrup (HFCS)
Dark corn syrup	Karo	Produced by ACH Food Companies Inc., USA Imported and marketed by Newage Gourmet Foods, Delhi	Import: June 2017	14 October 2019	6172005 010 Importer: 0210280 1	USA	Corn syrup, refiners syrup
Pancake syrup original	American Garden	A product of American Garden Co., New York Imported by Bajoria Foods Pvt. Ltd, Mumbai, Maharashtra	March 2018	24 months	1727350 510	USA	High fructose corn syrup (HFCS), corn syrup

Food product/ category	Brand/ company	Manufacturer/distributor/importer	Manufacturing /import date	Expiration date	Batch number	Country of origin	Relevant ingredients
					importer: B060		
Infant foods							
Infant formula	Similac IQ+ 1	Manufactured by Abbot Manufacturing Singapore Private Limited, Singapore (A subsidiary of Abott Laboratories, USA) Imported and marketed by Abbott Healthcare Pvt. Ltd, Mumbai	24 October 2017	17 April 2019	1030497	Singapore	Soy oil
Soy infant formula (lactose-free infant milk substitute, soy infant formula)	Similac Isomil (up to 24 months)	Manufactured by Abbott Laboratories BV Netherlands Imported and marketed by Abbott Healthcare Pvt. Ltd, Mumbai	16 February 2017	16 February 2020	P962AO74175NR	Netherlands	Hydrolysed corn starch, soy protein isolate (15.61%), soy oil
Follow-up formula for older infants	Enfamil A+ 3 (from 12 to 24 months)	Imported by Mead Johnson Nutrition India Pvt. Ltd, Mumbai, Maharashtra	21 August 2017	21August 2019	TJ7HNV5DC507759	Thailand	Vegetable oil, low erucic rapeseed oil, corn syrup solids, corn oil
Hypoallergenic formula	Nutramigen LGG	Manufactured by Mead Johnson, Netherlands Imported by Mead Johnson Nutrition (India) Pvt. Ltd, Mumbai, Maharashtra. LGG is a registered trademark of Valio Ltd, Finland	23 September 2017	23 September 2019	HL7JOC5C	Netherlands	Corn syrup solids, soybean oil, modified corn starch
Lactose and sucrose free infant milk substitute	Dexolac Nusobee Soya (up to 24 months)	Manufactured and marketed by Nutricia International Private Limited, Punjab (A subsidiary of Danone)	May 2017	August 2018	1705236911	India	Soya oil, soya protein isolate
Follow-on milk	SMA pro 2	Manufactured by SMA Nutrition (Registered Trademark of Societe des Produits Nestle SA)	14 August 2017	14 August 2019	7226080622	Ireland	Emulsifier (soy lecithin), rapeseed oil
Hypoallergenic infant formula	Similac Alimentum (from birth onwards)	Manufactured by Abott Laboratories Imported and marketed by Abbott Healthcare Pvt. Ltd, Mumbai	1 May 2017	1 June 2019	77477Z200	USA	Maltodextrin, soy oil, emulsifier modified corn starch
Lactose and sucrose free infant milk substitute, type II	Zerolac	Manufactured by Raptakos Brett & Co. Ltd, Mumbai, Maharashtra	February 2018	August 2019	D180074	India	Soya protein isolate (16.2%)
Protein supplements							
Protein supplement for adults	Prosure (delicious banana flavour)	Manufactured by Abbott Laboratories, Spain Imported and marketed by Abbott Healthcare Pvt. Ltd. Mumbai	22 September 2017	22 September 2019	81290QU	Spain	Soya oil, soya polysaccharide, soya lecithin
Soybean-based nutritional beverage mix	Protinex (elaichi flavour, low fat)	Manufactured and marketed by Nutricia International Pvt. Ltd, Punjab (Protinex is a registered trademark of Danone Asia Pacific Holdings PTE Ltd)	August 2017	15 Months	ZPEQ002527	India	Soya protein isolate, corn flour

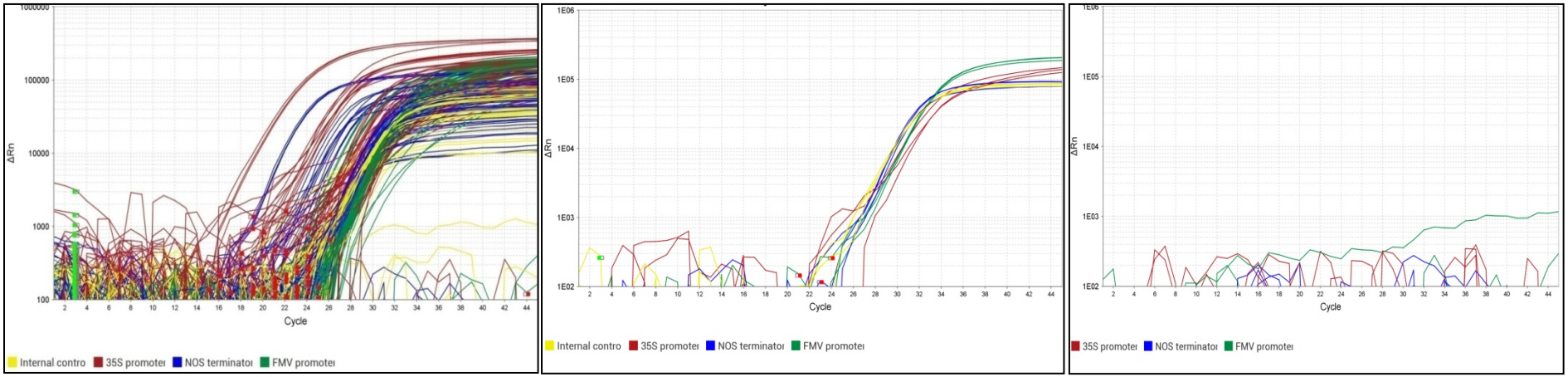
*'-' denotes no labelling w.r.t. GM, NA: Not Available; *considered imported as seeds are from Australia*

Annexure 2: qPCR amplification plots for GM markers in food and control samples



a **b** **c**

Figure 2 A: qPCR linear amplification plots for (a) GM positive samples (b) positive control and (c) negative control samples.



a **b** **c**

Figure 2 B: qPCR logarithmic amplification plots for (a) GM positive samples (b) positive control and (c) negative control samples.

Annexure 3: List of abbreviations of unit symbols used in sections 2.2 and 2.3

Symbol	Unit
g	gram
ml	milli litre
µl	micro litre
°C	degree Celsius
min	minute
s	second
%	per cent
x g	relative centrifugal force
U	Unit
nm	nanometer
rpm	revolutions per minute