



Detection of Genetically Modified Additives in Meat Products in Riyadh City

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Abstract

Vegetable proteins such as soybean protein have numerous nutritional and functional characteristics, and consequently, their utilization in meat products development has dramatically increased in recent decades. Due to high demands for soybean, transgenic Roundup Ready (RR) soybean line grains were developed and widely distributed into global markets. The current study was designed to investigate the presence of transgenic soybean in meat products sold in Riyadh food retails, Saudi Arabia. After extraction of DNA from meat product samples, qualitative duplex polymerase chain reaction (PCR) was used to detect the genetically modified (GM) soybean products in the meat samples using pairs of primers targeting the lectin gene and the 35S promoter. Real-time PCR was used to quantify the percentage of RR soy products in the positive samples. The results clarified that out of 96 tested meat product samples (minced, burger, luncheon, canned, and sausages), 75 samples were positive for the presence of lectin gene, of which 42 samples representing 43.75% of total meat product samples were positive for the presence of 35S promoter. All positive samples for 35S promoter contained RR soy below 0.1%. The results of the consumer acceptance questionnaire of GM additives in meat products proved the presence of several critical aspects of concerns to consumers of meat products in different localities of Riyadh city.



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Introduction


Soybean is a unique food crop that received significant attention and wide application in meat products in recent years due to its extraordinary functional and nutritional attributes. Due to

high demands for nutritious and technically acceptable food ingredients to feed the increased global population, the production and utilization of genetically modified organisms (GMO) spread widely in the world. Of these GMOs, the genetically

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modified soybean, namely the Round up Ready (RR) soybean, has been applied in many foods. To date, many countries still have concerns about the production and consumption of GMOs - containing foods, despite the improved yield and nutritional quality of GMOs.¹

The first approval of GM was in 1996, and since that, time GM soybean has become the dominant crop in USA and Argentina^{2,3} and its production was raised from 7.4% in 1996 to 75% in 2002 in the USA. The key to the successful production and marketing GM foods is consumer acceptability. The consumer concerns about GM foods are likely due to many factors such as religious and ethical concerns, possible allergic responses, insufficient labeling, and the development of antibiotic-resistant genes.⁴ Accordingly, health governmental and nongovernmental organizations put increased efforts to make tight regulations and clear GM labeling foods in various regions of the globe. USA is one of the top producers of GMO, however, GM labeling in this country is still not mandatory, whereas it is mandatory in many Asian and European countries.⁵

The Arabian countries' imports of food products are increasing; however, it is difficult to prohibit and control the imported food containing GM ingredients. On the other hand, consumption of GM food or food containing GM additives is still questionable from a public health point of view, especially in countries that depend upon the importation of food products where its control systems are not well developed to control the biosecurity of these food product imports. Consequently, strict examinations, risk analyses, and detection of GM additives in food products are of particular concern.^{6,7}

Genetically modified food is becoming an important issue globally. In Saudi Arabia, importing GM foods was approved in 2005 however, importing GM animal products, dates, grains and seeds and ornamental plants were excluded from the approval. In addition, clear labeling in both Arabic and English of GM materials and official certification indicating the approval of human consumption of these foods in the country of origin is required.⁸ In this regard, Ahmed *et al.*⁹ surveyed 202 samples for genetically modified food in Saudi Arabian markets and found that out of 20 positive meat product samples,¹⁶ were ground

minced meat; however, the source of the GMO in these samples was the added soybean.

Introducing GM foods into the global markets has led to the development of numerous regulations. In this sense, the European Union has set regulations, namely EC Regulations No. 1829 and No.1830 that are mainly set for controlling the release of GMO into the environment and for the labeling and tracing food and animal feed that contain GMOs, respectively.¹⁰⁻¹¹ Many countries also introduced regulations for labeling GM foods within and outside the European Union, and the latter indicated that labeling GM foods with more than 0.9% GMO is mandatory. From the consumer's perspective, GM-containing foods should be labeled to allow the consumer to decide whether to buy or not buy these foods. The extensive application of soybean protein in meat product formulations is likely because soy protein has several functional features, namely emulsification properties, water and oil binding capacities, textural properties, and sensorial attributes, besides its low cost compared to other animal-based and synthetic emulsifiers.¹¹ Furthermore, soybean is also used as a fat replacer in meat products to meet the consumer needs for natural, safe, and healthier food products.¹²

The main transgenic soybeans permitted for trading in the European Union countries is Round up Ready (RR) soybean. For the tracing of RR soybeans in food products, numerous detection and identification methods were developed. The main methods used to detect RR soybean in raw or processed food products are protein-based and DNA-based methods¹³⁻¹⁵ The first method the EPSPS gene products are detected,¹³ where as in the later genes were detected using both quantitative and qualitative polymerase chain reaction (PCR) methods.^{14,15} The most commonly used methods for detecting GMOs in food products is the polymerase chain reaction (PCR) method.¹⁶ The research targets of this work aimed at addressing the existence of genetically modified soybean ingredients in meat products in Riyadh city through the following aspects: Application of a Consumer Acceptance Questionnaire regarding GM soybean additive in meat products in Riyadh city. In addition, means of detecting GM soybean additives in meat products.

Materials and Methods

Materials

Meat Product Samples Sources And Collection

Ninety-six samples of meat products of different producing companies were collected randomly from Riyadh local markets.

At least 1 kg of all samples was collected from places of display and marketing in Riyadh local markets. Samples were taken cleanly, without opening or removing packaging materials, it immediately transported separately in clean containers. Samples were kept at 4°C until analysis.

The collected samples were 22 minced meat samples (13 local and 9 imported), 21 burger meat samples (14 local and 7 imported), 22 luncheon meat samples (15 local and 7 imported), 12 canned meat samples (7 local and 5 imported) and 19 sausages meat samples (11 local and 8 imported). The extracted DNA from these samples was analyzed using duplex

PCR and the primers used were lectin gene and 35S promoter.

Assessment of Consumer Acceptance of Gm Soybean Additive in Meat Products

The study assumes a lack of awareness among consumers, and the presence of factors that affect consumers' acceptance of genetically modified GM foods. The survey aims to assess awareness and measure consumer acceptance of GMFs, and in order to recognize the reasons that influence the consumer acceptability of GM additives in meat products.

Questionnaire Designing

we have designed a questionnaire (Table 1), The questionnaire includes a study of four main aspects: 1) Measuring awareness about GMFs, 2) Assessment of consumer acceptance of GMGs, 3) The effect of health risks on the acceptance of GMGs, 4) Assessment of the effect of the economic factor on GMGs acceptance, 5) labeling importance

Table 1: Consumer Acceptance Questionnaire of GM additives in Meat Products

Question	Comments	Localities Responders (Number and Percent)			
		1 East	2 (west)	3 (North)	4 (South)
Consumer Sample Size		200	200	200	200
A- How well were you informed about GMO in meat products before this questionnaire?	* Very well * Somewhat * Not informed				
B- GM soybeans in meat products contain genes while non-GM soybeans do not.	* True * False * Don't know				
C- A person's genes could be altered by eating GMO in meat products.	* True * False * Do not know				
D- How safe or risky are GMO in meat products to human health?	* Risky * Neither * Safe				
E- How willing are you to consume meat products containing GMO ingredients?	* Don't know * Very willing * Somewhat * Not very * Would avoid				
F- How important is it to label meat products containing GMO ingredients?	* Very * Somewhat				

G- What type of labeling would you support?	<ul style="list-style-type: none"> * Not very * Mandatory for GM and non- GM * Mandatory for GM * Voluntary * Don't support any
H- Willing to pay less money for meat products containing GMO ingredients	<ul style="list-style-type: none"> * Very willing * Somewhat * Would avoid

Study Samples

The questionnaire was distributed it between 800 meat product's consumers in some common supermarkets in each of four different localities east, west, north, and south (200 consumers from each location) of Riyadh city, in order to ensure that the sample represented the study population, to obtain a comprehensive view about awareness and the degree of accepting GMFs and the effected factors. And since the study was conducted in the markets, most of the respondents are the people responsible for purchasing foodstuffs, and this includes both males and females.

Data collection

The questionnaire was conducted by conducting personal interviews, in which the objectives of the study were clarified and the paper questionnaire was distributed to the study samples to fill out their responses, or questions were asked to them and filled out by the researcher.

The questionnaire responses were collected for each of the four different regions separately and entered into the Excel program for processing in preparation for analysis.

Statistical Analysis

Date of the questionnaire were collected and treated by Excel, the statistical analysis were performed using SPSS V. 22, the data were expressed as percentages of the responses frequency and the mean of the scored responses by given 5 for the highest values and 1 for the lowest for all questionnaire items.

Detection of Soybean Additive in Meat Products Reference Materials

In this study, RR soybean was used as a positive control, and Bt-11 maize was used as the negative

control. Both certified standard materials were obtained from Institute for Research Materials and Measurements (Geel, Belgium), and 0%, 0.1%, and 1% concentration of each control was prepared using powder of RR soybean and Bt-11 maize.

DNA Extraction

The extraction of DNA from meat products was carried out as described earlier²² with some modifications. Briefly, 400 mg sample was added to 3 mL of cetyl trimethyl ammonium bromide (CTAB) buffer and then homogenized using a homogenizer. After that, 1 mL homogenate was subjected to CTAB precipitation method for the purification of DNA.²³ For the extraction of DNA from reference materials, about 100 mg sample was extracted using the same method. The extraction of both samples and reference materials was carried out twice. The concentration and purity of the extracted DNA were assessed using an Ultraspec 2000 spectrophotometer (Pharmacia Biotech, USA) at 260 and 280 nm. Known concentration (25 ng/ μ l) of calf thymus DNA was used as a control for assessing the concentration of extracted DNA.

Duplex PCR Assay

The presence of native soybean and RR soybean was measured using duplex PCR assay. In this assay, two set of primers were used to identify native soybean DNA and GM soybean DNA that includes lectin gene primers⁶ and 35S promoter primers.²⁴ For duplex PCR assay, approximately 100 ng extracted DNA was mixed with 25 μ l of PCR mixture containing the followings; 2.5 μ l of reaction buffer, 1.5 mM of magnesium chloride, 0.2 mM dNTPs, 0.1 μ M lectin primers, 0.6 μ M 35S promoter primers, 1 unit Taq native polymerase (Fermentas, Vilnius, Lithuania) and MilliQ water to complete the volume to 25 μ l. For the amplification

of target DNA, 40 cycles of 25 s denaturation at 95°C, 30 s primer annealing at 60°C, 45 s extension at 68 °C, and 10 min final extension at 68 °C were run using a Master cycle rep gradient S thermocycler (Eppendorf, Hamburg, Germany). To accurately identify target DNA materials, non-template, positive, and negative controls were used in the PCR tests. Known concentrations (0, 0.5, and 1%) of RR soybean reference materials were used to check the sensitivity and robustness of the applied method.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was applied to determine the PCR products. The gel of 2% agarose and 0.5 g/mL ethidium bromide was prepared and run using an agarose electrophoresis unit and TE buffer. PCR products and a 50 base pair gene leader (Fermentas, Vilnius, Lithuania) were run. Bands of target DNA, reference materials, and leaders were visualized using a UV transilluminator. The images were captured with DOC PRINT system (ViberLourman, USA).

Real-Time PCR

For accurate quantification of the percentages of RP soybean in GM positive samples, a real-time PCR procedure was carried out using lectin gene and RR soybean (35S promoter) gene primers as described by Taski-Ajdukovic *et al.*²⁵ with slight changes. The lectin gene probe, forward primer, and reverse primers were 5/-Tx-Red-CTC-TTG-GTC-GCG-CCC-TCT-ACT-CCA-C-BHQ2-3/, 5/-CGG-CAC-CCC-AAA-ACC-C-3/, and 5/-CGT-ACC-GGT-TTC-TTT-GTC-CCA-3/, respectively. While the probe, forward primer, and reverse primer of RR soybean were 5/-FAM-CCT-TCA-TGT-TCG-GCG-GTC-TCG-C-BHQ1-3/, 5/-CAT-TCC-CGG-CGA-CAA-GTC-3/, 5/-TTG-ATG-ACG-TCC-TCG-CCT-TC-3/, respectively. The samples were mixed with primers and iQTM Supermix (Bio-Rad, Hercules, CA, USA) and amplified using an iCyclerIQ multicolor real-time PCR (Bio-Rad, Hercules, CA, USA) that programmed as follow: 2 min initial denaturation at 95 °C, followed by 15 s denaturation at 95 °C, and 50 s annealing and extension at 60 °C for a total of 45 cycles.

Table 2: Consumer Acceptance Questionnaire of GM additives in Meat Products

Question	Comments	Localities				Total Mean
		East	West	North	South	
N		200	200	200	200	
		%	%	%	%	
A- How well were you informed about GMO in meat products before this questionnaire?	well	1	8	20	2	7.75
	Somewhat	88	68	77	94	81.75
	Not informed	11	24	3	4	10.5
B- GM soybeans in meat products encompass genes while non-GM soybeans do not.	TRUE	6	3	6	15	7.5
	FALSE	85	63	78	18	61
	I don't know	9	34	16	67	31.5
C- A person's genes could be altered by eating GMO in meat products.	TRUE	6	5	16	13	10
	FALSE	70	78	15	62	56.25
	I don't know	24	17	69	25	33.75
D- How safe or risky are GMO in meat products to human health?	Risky	11	8.5	15	22	14.125
	Neither	44	57	54.5	54	52.375
	Safe	45	34.5	30.5	24	33.5
E- How willing are you to consume meat products containing GMO ingredients?	I don't know	0	5.5	11.5	16	8.25
	Would avoid	10	36.5	3	19	17.125
	Somewhat	34	43	10.5	45.5	33.25
	Not very	38	12.5	57.5	18.5	31.625
	Very willing	18	2.5	17.5	1	9.75
F- How important is it to label meat	Very	84	49	60	79	68

products containing GMO ingredients?	Somewhat	13	29	21	19	20.5
	very	3	22	19	2	11.5
G- What type of labeling would you support?	Obligatory for GM and non- GM	48	39	30	67	46
	Obligatory for GM	48	37	52	27	41
	Voluntary	3	20	17	4	11
	Don't support any	1	4	1	2	2
H- Willing to pay less money for meat products containing GMO ingredients.	Very willing	27	54	27	64	43
	Somewhat	44	38.5	50.5	27	40
Would avoid	Would avoid	29	7.5	22.5	9	17

Table 3: The Score of Knowledge, Acceptability, Health risk factor, Economic factor and Labels

Items	QN	Response	Score
Knowledge	A	Not informed	.1050
		Somewhat well	2.4525
		Total	.3875
	B	FALSE	2.9450
		I don't know	.6100
		TRUE	.9450
	Total	.3750	
Total	1.9300		
Total	2.4375		
Acceptability	E	I don't know	.2475
		Not very	1.2650
		Somewhat	.6650
		Very willing	.0975
		Would avoid	.8563
	Total	3.1313	
Health risk factor	C	FALSE	.5625
		I don't know	1.0125
		TRUE	.5000
	Total	2.0750	
	D	Neither	1.5713
		Risky	.7063
		Safe	.3350
Total	2.6125		
Total	2.3438		
Economic factor	H	Somewhat	1.2000
		Very willing	2.1500
		Would avoid	.1700
	Total	3.5200	
Labels	F	Somewhat	.4100
		Not very	.1150
		Very important	2.0400
	Total	2.5650	
	G	Don't support any	.0200
		Obligatory for GM	1.6400
		Obligatory for GM and non- GM	2.3000
Voluntary		.3300	
Total	4.2900		
Total	3.4275		

Results and Discussion

Consumer Acceptance of Gm Soybean Additive in Meat Products

The results of the consumer questionnaire regarding the acceptance of GM additives in meat products are present in Table 2 and Table 3. The results showed that low rate of the consumers were well known about GMF, most of them were known somewhat, and the rest 10.5% were Not informed. Similarly their knowledge about soybeans in meat products encompass genes or not. And 10% of the responds were agree that person's genes could be altered by eating GMO in meat products, while 56.25% were not and 33.75% were don't know. Also 14.125 of the responds were think that the GMO in meat products to human health is risky, and 52.375% of them were don't know, while 33.5% were think it safe. A rate of 17.125% of the responds were would avoid the GMO, and only 9.75% were very willing, and 33.25%, 31.625% were somewhat not very willing to consume GMO respectively. Most of consumers believe that it is very important to label meat products containing GMO ingredients, and the obligatory for GM and non- GM. A percentage of 43% were willing to pay less money for meat products containing GMO ingredients, and 40% some what and 17% would avoid GMO. The results in Table 3 showed the score of knowledge, acceptability, health risk factor, economic factor and labels, the results showed that all means of score were found to be less than 3 except that of acceptability 3.13125 which considers very weak, and the importance of labels information of GMF was higher 3.52, also economic factor that affect GMF consumption was also increased 3.4275.

Extraction of Dna From Meat Products

The applied CTAB extraction method resulted in DNAs with a ratio of OD260 nm/OD2800 nm of 1.8 to 2.0 from all samples indicating the high quality of the extracted DNA. The pure DNAs were used as templates for amplifying, identifying, and quantifying RR soybean genes using PCR methods (duplex and real-time PCRs).

Duplex PCR

In this study, duplex PCR analysis was used for screening of GMOs in meat products because in this type of PCR, two genes could be amplified

in the same time, which is not possible in most normal PCR analyses of GMOs. This advantage of using duplex PCR in the analysis of GMOs in food products could likely reduce the costs and analysis time, and hence rapid tracing and decision on trading and consumption of GM foods.²⁶ In the duplex PCR and agarose gel results, amplicons with the size of 118 and 195 bp were observed, which indicated the amplification of soybean specific gene (lectin) and RR soybean gene (35S promoter), respectively. Specific amplification of the lectin gene (118 bp) suggested that the applied CTAB extraction method is suitable for extracting and purifying soybean additives from meat samples. In contrast, the amplification of 35S promoter gene (195 bp) indicated the existence of transgenic materials in the meat samples. In PCR analysis of specific soybean genes, amplification of these genes is important to differentiate between negative and positive results,²⁷ and thus in the current study, the identification of these genes indicated the potential of duplex PCR for analysis of GMOs in meat products.

Our results for the 96 meat product samples are present in Table 4 & Figures 1-5. The existence of the lectin gene was recorded in 75 samples, out of which 42 samples representing 43.75% were positive for 35S promoter, the indicator for GM Roundup Ready soybean. The presence of lectin in 75 samples (Table 4), clarifying that 78.13% of examined meat samples contain soybean additives, while 21.87% of tested meat samples do not. In agreement with these findings, previous reports indicated the detection of the lectin gene in sausage,²⁸ and processed meat²⁹ samples.

Quantitative Real-Time Pcr of Meat Samples

Quantitative real-time PCR approaches with primers specific for RR soybean were used to analyze the amount of GM additive in meat product samples that showed positive results on duplex PCR analysis. The results showed that all the tested meat samples contain less than 0.1% RR soybean that is significantly lower than the threshold limit (0.9%). Our results regarding the consumer acceptance questionnaire of GM additives in meat products (Table 3) proved the presence of several critical aspects of concerns to consumers of meat products in different localities of Riyadh city.

Several surveys have been carried out to screen the existence of GMOs in food systems. El-Sanhoty *et al.*³⁰ recorded that 20% of tested Egyptian soybean samples contained RR soybean. However, Oraby *et al.*³¹ examined 24 different food samples in Egypt and found three positive products for 35S promoters. In Malaysia, Abdullah *et al.*³² conducted a study on the processed food in Malaysian markets to detect GM soybean. They found 18 positive samples out of 85 tested samples. In Turkey, Aril and Cakir³³

(2008) reported genetically modified soybean products in almost all tested soybean food stuffs. In another study in Serbia, analysis of fifty processed meat products for GM soybean ingredients indicated that 24% (12 samples) of them has 35S promoter genes. All these positive samples contain RR soybean.²⁵ Furthermore, a study on 35 food samples of canola, soybean, and corn that have high saleable significance in Canada showed that about 33% of the samples were GM.³⁴

Table 4: Analytical results of genetically modified soybean additive in local and imported meat products using duplex PCR

Meat Product	Number of samples	Negative for lectin gene%	Positive for lectin gene%	Positive for 35S promoter%
Minced (local)	17	47.06	52.94	23.53
Minced (imported)	10	20.00	80.00	70.00
Burger (local)	12	16.67	83.33	25.00
Burger (imported)	9	0.00	100.00	66.67
Luncheon (local)	12	25.00	75.00	33.33
Luncheon (imported)	10	30.00	70.00	60.00
Canned (local)	8	25.00	75.00	37.50
Canned (imported)	5	0.00	100.00	80.00
Sausages (local)	9	11.11	88.89	22.22
Sausages (imported)	4	0.00	100.00	75.00
Total	96	21.88	78.13	43.75

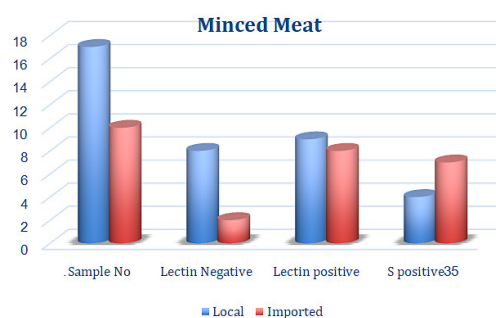


Fig. 1: Prevalence of genetically modified soybean additives in examined meat products

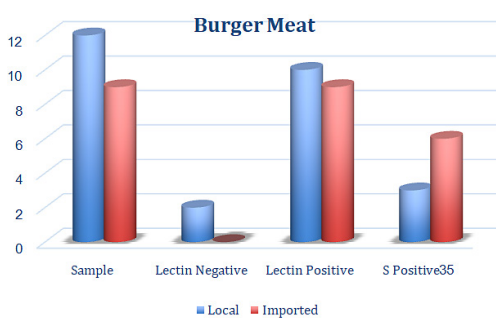


Fig.2

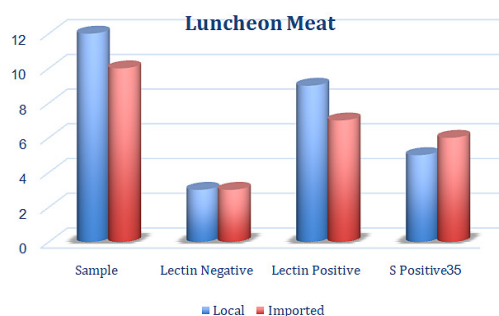


Fig.3

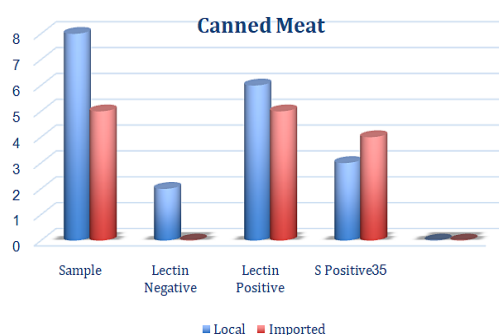


Fig.4

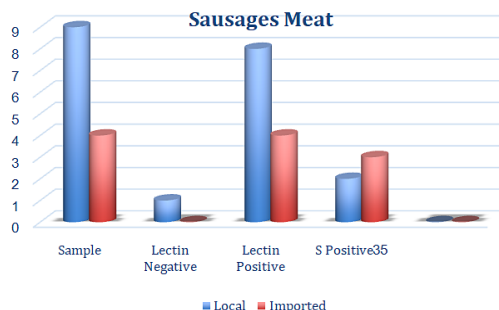


Fig.5

In Saudi Arabia, the regulations for considering the food to be GM and labeled are set as 1% maximum of threshold limit. Consequently, the label should contain a triangle shape in which a statement of this product contains GM products should be written. Due to the global concerns about GMOs in foods, the Ministry of Agriculture in Saudi Arabia in 2004 has banned imports of genetically modified seeds. Consequently, there is no GM crop in the country. Recently, the Saudi Food and Drug Authority have permitted the imports of GM containing processed plant and vegetable foods if they have clear label.³⁵

Conclusion

Our results demonstrated the presence of genetically modified RR soybean in 43.75% of examined meat products commercially available in Riyadh city; however, all were below 0.1%, and labeling was not legally necessary. On the other hand, the addition of soybean additives, regardless being genetically modified or not, to meat products without labeling contradict consumer rights to be announced with the constituents of meat products he is going to buy, the matter that could be considered a mean of commercial adulteration. However, the consumer acceptance questionnaire of GM additives in meat

products proved the presence of several critical aspects of concerns to consumers of meat products in different localities of Riyadh city.

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Conflict of Interest

The author declares no conflict of interest.

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