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Research Article

PCR Detection of Soy Protein in Ready to Eat Meat Doners [1]

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Abstract

Addition of soy protein sources in food products is widely used because of their functional properties such as water binding, fat binding, beneficial effects on texture and emulsification capability and providing improved economy with increasing yield. However, the use of soy protein in food products causes economical disadvantages because of replacement of an expensive ingredient like meat with a cheaper ingredient like soy and health risks for the consumers as well. Soy is an important allergy source for sensitive consumers. Because of these reasons, the most recent meat products regulation of Turkish Food Codex has banned the addition of soy in doners since 2012, like several other countries. Detection of soy in food products is performed by detection of soy protein or soy DNA. Because DNA is more stable to processing, PCR methods are shown to be more reliable when used in processed foods. In our study, 50 doner samples were collected from various retail sales points. Twenty-five doner samples were collected before and 25 after the regulation was enacted. DNA was isolated from doner samples and PCR testing of these DNA extracts were performed. The detection results of the doner samples showed that any of the 25 samples collected after the regulation enacted did not contain soy ingredient while 3 of 25 sample (12%) collected before the regulation came into force contained soy.

Keywords: *Soy, Doner, PCR, lectin, GM soy*

Tüketime Hazır Dönerlerde PCR ile Soya Proteininin Aranması

Özet

Soya proteinleri, gıda endüstrisinde, su bağlama, yağ bağlama, tekstür ve emülsifikasyon yeteneği, verim arttırma gibi fonksiyonel özelliklerinden ve verimin artışına bağlı olarak ekonomik karlılığı arttırmasından dolayı geniş kullanım alanı bulmaktadır. Ancak gıda ürünlerinde soya kullanımı, tüketiciler için ekonomik kayıplar ve sağlık riskleri gibi olumsuzluklara sebep olmaktadır. Soya, hassas tüketiciler için önemli bir alerji kaynağıdır. Bu gibi nedenlerden ülkemizde Aralık 2012'de çıkan Türk Gıda Kodeksi Et ve Et Ürünleri Tebliği ile dönerlerde soya kullanımı birçok diğer ülkelerde de olduğu gibi yasaklanmıştır. Gıdalarda soyanın tespit edilmesi soya proteinin ya da soya DNA'sının tespit edilmesi ile gerçekleştirilir. Ancak, DNA gıda işleme şartlara daha dayanıklı olması sebebiyle işlenmiş gıdalarda çok daha güvenilirdir. Çalışmamızda, 25 adedi Et ve Et Ürünleri Tebliğinin yürürlüğe girmesinden önce, diğer 25 adedinin ise tebliğin yürürlüğe girmesinden sonra olacak şekilde 50 adet döner örneği çeşitli perakende satış noktalarından toplanmıştır. Döner örneklerinden DNA izole edilmiş ve bu DNA'ların PZR testleri gerçekleştirilmiştir. Elde edilen sonuçlara göre Et ve Et Ürünleri Tebliğinin yürürlüğe girmesinden sonra toplanan hiçbir örnekte soya tespit edilemezken, tebliğin yayınlanmasından önce toplanan 25 örnekten 3'ünde (%12) soya varlığı saptanmıştır.

Anahtar sözcükler: *Soya, döner, PCR, lektin, GD soya*

INTRODUCTION

Addition of non-meat protein sources in food products, is widely used because of it is capability of improving the product properties and reducing the production cost. Soy protein fractions are preferred because of their higher protein content and functional properties [1,2]. The use of soy protein fractions in meat products is also widely applied for their properties such as water binding, fat binding, texture and emulsification capability and providing improved economy with increasing yield [1-3]. Soy protein fractions are available in various forms such as; flour, grits, concentrates, isolates and textured [3]. However, the use of soy in food products, causes health and economic risks for the consumers as well. It brings economical disadvantages because of replacement of an expensive

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ingredient like meat with a cheaper ingredient like soy. Besides soy is an important allergy source for sensitive consumers [3-5]. Because of these reasons, use of soy in food products are limited or banned in various countries [4,5]. In our country presence of soy in a food product has to be declared in the label. Additionally, meat products regulation of Turkish Food Codex banns the addition of soy in doner [6]. Despite, addition of soy in ready to eat meat products like doner, meat patties is not uncommon for reducing the cost.

Several methods have been used for detection of soy in food products so far [3,4,7]. However, most reliable methods are based on detection of soy protein or DNA. Protein based methods includes, electrophoretic or serologic (ELISA) methods [7-9] while PCR is most widely used as DNA based methods [4,10]. Because DNA is more stable to processing conditions than protein, PCR methods are shown to be more reliable when used in processed foods [10].

In this study, the presence of soy in doner kebabs sold in local sales points were investigated. The doner samples examined were purchased before and after the regulation enacted, to evaluate the effect of regulatory enforcement. For this, PCR detection of soy specific *lectin* gene with PCR was performed. The positive samples were further analyzed to detect whether they are Genetically Modified (GM) or not.

MATERIALS and METHODS

Doner Samples

For the study, 50 meat and poultry doner samples were collected from various retail sales points. Twenty five of these doner samples were collected before the regulation was released, while 25 were collected after the regulation. Additionally, soybean powder (IRMM, Geel, Belgium) and beef were used as positive and negative controls respectively. All the meat samples were stored in -20°C freezer till they were used.

DNA Extraction and Purification

For DNA isolation from doner samples and positive and negative control samples, the Promega Wizard™ DNA isolation kit (Promega, Madison, USA) was used according to the manufacturer's instructions. Two hundred to three hundred milligrams of food material taken from a previously homogenized sample was mixed with 860 µl of extraction buffer (10 mM Tris-OH, 150 mM NaCl, 2 mM EDTA and 1% w/v sodium dodecyl sulfate), 100 µl of guanidine hydrochloride (5M) and 40 µl of proteinase K (20 mg/ml), then incubated at 65°C overnight. The samples were then centrifuged at 13.500 g for 10 min. After centrifugation, 500 µl of the supernatant was mixed with 1 ml of Wizard™ resin (Promega, Madison, USA) and pushed through a Wizard™ minicolumn (Promega, Madison, USA). The column was further washed with 2 ml of isopropanol. Following centrifugation of the column at 12,000 g for 5 min, the DNA was eluted with 50 µl of pre-warmed (65°C) elution buffer (10 mM Tris-OH). The columns were incubated at room temperature for 1 min and centrifuged at 10.000 g for 2 min. The collected DNA was stored at -20°C until used.

DNA quantification was achieved by measuring the UV absorption at 260 nm using a T80 UV/VIS spectrometer (PG Ins. Ltd., UK).

PCR Primers and PCR Conditions

The primers GMO3 (5'-GCC CTC TAC TCC ACC CCC ATC C-3') and GMO (5'-GCC CAT CTG CAA GCC TTT TTG TG-3') were used for the amplification of soy-specific *lectin* sequence and yielded a longer PCR product (118 bp) [10,11]. The primers 35s-f2 (5'-TGATGTGATATCTCCACTGACG-3') and petu-r1 (5'-TGTATCCCTTGAGCCATGTTGT-3') were used for the amplification of GM soy-specific Round Up Ready (RUR) soy sequence and yielded a longer PCR product (172 bp) [11]. All PCR reactions were performed with a CG Palm-Cycler (CG 1-96 Genetix Biotech, Australia & Asia).

Amplification reactions for *lectin* contained; 5 µl of genomic DNA and 20 µl of the appropriate PCR reaction mixture. PCR reaction mixture consisted of 1X buffer (Fermentas), 1.5 mM $MgCl_2$ (Fermentas), 0.2 µM of each primers, 0.8 mM of each dNTP (Fermentas) and 0.5 IU of Maxima™ Hot Start *Taq* polymerase (Fermentas). The amplification profile used for this mixture was as follow: denaturation for 10 min at 95°C; amplification for 30 s at 95°C, for 30 s at 60°C, for 60 s at 72°C; number of cycles 35; final extension for 3 min at 72°C.

For detection of GM soy, amplification reactions which consisted of; 1X buffer (Fermentas), 1.5 mM $MgCl_2$ (Fermentas), 0.2 µM of each primer for RUR soy amplifications, 0.8 mM of each dNTP (Fermentas) and 0.5 U of Maxima™ Hot Start *Taq* polymerase (Fermentas) were used. The amplification profile used for this mixture was as follow: denaturation for 10 min at 95°C; amplification for 30 s at 95°C, for 30 s at 60°C, for 25 s at 72°C; number of cycles 40; final extension for 3 min at 72°C.

PCR products were electrophoresed through a 2% agarose gel containing ethidium bromide. As a size reference, a 50 bp DNA ladder (Fermentas) was used. Visualization of the gels was performed with a UV trans-illuminator, and the gels were captured with the Dolphin-DOC system and Dolphin 1D Gel analyzing software (Wealtec, Nevada,USA).

RESULTS

Total of fifty commercially sold ready to eat doner samples which 25 of them were collected before the related regulation came in to force while the rest 25 were collected after the enforcement were detected for

presence of soy protein with PCR in the present study.

For ensuring the reliability of the detection tests appropriate quality control studies were performed throughout the whole study.

For confirmation of the specificity of the primers PCR tests were performed with DNA extracts obtained from soybean powder and beef. The results showed that the primers were specific to soy and did not give any false result with the other main ingredient of doner like beef *(Fig. 1)*.

False positive results related to carry over contamination during DNA sampling and extraction were avoided by processing sterile milli Q water in parallel with the samples at each step of extraction and PCR [12].

For elimination of false negative results related to PCR inhibitors that might be present in the sample, DNA extracts of each sample were run in triplicate for each PCR reaction which one of the extracts were spiked with soybean powder DNA while the other were run without spiking. The results showed that any of the 50 samples did not contain any inhibitor.

The detection results of the doner samples showed that 3 of all 50 samples (6%) we analyzed were positive for soy. Any of the 25 sample collected after the regulation released, did not contain soy ingredient while 3 of 25 sample (12%) collected before the regulation came into force contained soy. The gel electrophoresis results of the positive samples are given at *Fig. 2*.

The *lectin* positive samples were further analyzed for presence of GM soya. The results of this detection proved that any of these samples contained soy from a GM source.

DISCUSSION

There is not much study performed on detection

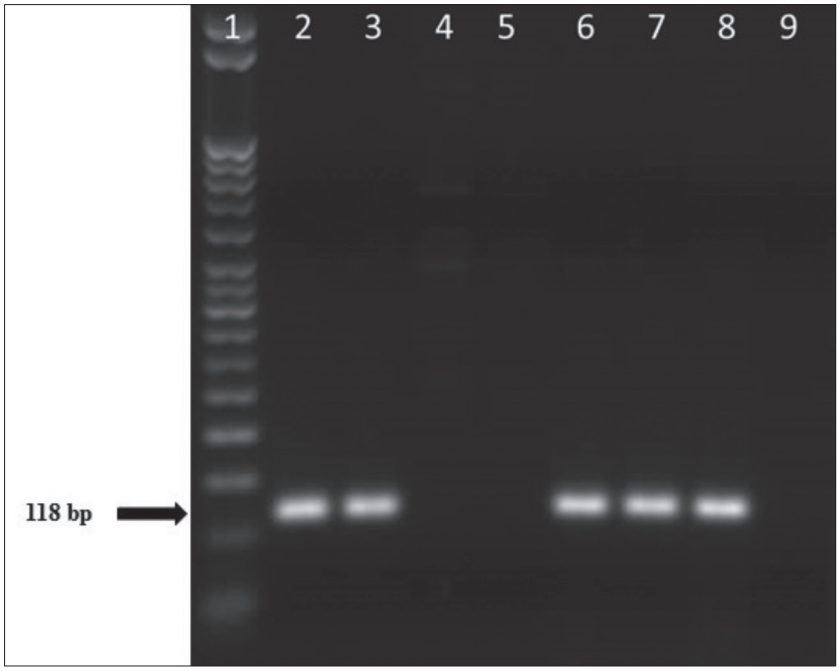


Fig 1. Specificity of primers

Lane 1: DNA ladder, Lane 2-3: soy flour, Lane 4-5: Beef, Lane 6-8: soybean powder, Lane 9: PCR mili q water

Şekil 1. Primerların özgünlüğü

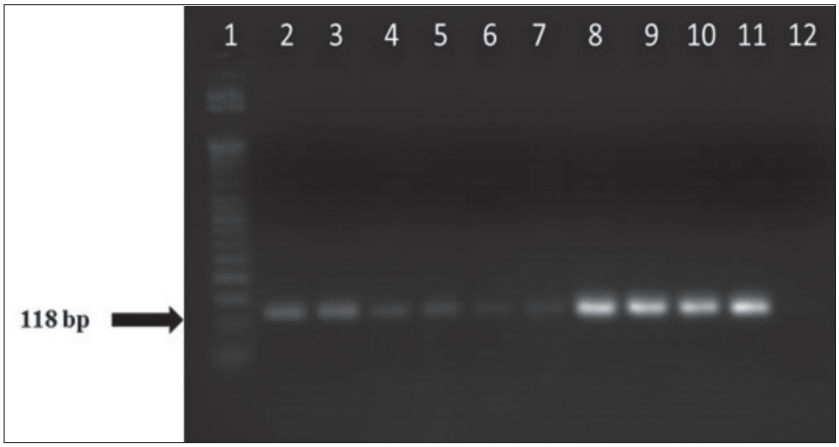
1. Sıra: DNA marker, 2-3. Sıra: Soya unu, 4-5. Sıra: Sığır eti, 6-8. Sıra: Soya fasulyesi tozu, 9. Sıra: PCR mili q su

Fig 2. Agar gel electrophoresis of positive doner samples

Lane-1: DNA ladder, Lane-2-3: doner sample-12, Lane-4-5: doner sample-13, Lane 6-7: doner sample-19, Lane 8-11: soybean powder Lane 12: PCR milli q water

Şekil 2. Pozitif döner örneklerin agar jel elektroforezi

1. Sıra: DNA marker, 2-3. Sıra: 12. Döner örneği, 4-5. Sıra: 13 Döner örneği, 6-7. Sıra: 19. Döner örneği, 8-11. Sıra: Soya unu, 12. Sıra: PCR milli q su



on soy in meat products. The main reason of this issue is that, it is not banned in several countries and it has been banned in our country only in 2012. For this purpose, few studies performed on detection of soy is mainly focused on detection of GMOs [13,14]. Ulca et al.[13] detected the presence of GM soy in various type of meat products and the samples of this study were collected recently after the regulation was released. According to these results, 32 out of 38 total samples contained soy and 2 of these positive samples were GM. In our previous study on detection of GM soya in food products which we performed before the regulation was enacted, we detected several meat products containing soy ingredients. Because it was legal, it was declared on the label as well [14]. However, different than our results (6% in our study) all of the doner samples Ulca et al.[13] analysed were positive for soy. According to the *Ct* results of this study, the soy level of these doner samples are quite low and most probably reflects trace amounts of presence. Thus, this difference might be related to the difference in the limit of detection of the two methods. The possible disadvantage of too low detection limit (below 0.1%) in authenticity testing is discussed by several authors because of its effect on discriminating technical unavoidable contamination and intentional addition [15,16]. For this purpose, it is not evaluated as a weakness of the method used in our study.

Based on the results of our study, we can conclude that intentional addition of soy was not commonly used in the case of doner even before the regulation. The level of usage has decreased after the regulation came in to force which showed that the producers comply with the regulation requirements. However, it is strongly recommended to further monitor the other type of processed meat products which are more commonly contained soy before the regulation.

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