Detection of Animal Species in Some Meat and Meat Products by Comparatively Using DNA Microarray and Real Time PCR Methods

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Summary

Determination of animal species in the meat and meat products is one of the interest of food science, it is also important for consumer rights and food safety. Increasing world population has also remarkably impacted the demand for meat and meat products. Based on this fact, issues related to safety and quality in the meat products have brought up in that manner as well. Through DNA based molecular methods are improved in food analysis, it is preferred increasingly in the control of food safety. In this study, a total of 73 samples of the meat and meat products sold in stores, meat selling markets and public bazaars located in different districts of İstanbul province were analyzed for the detection of animal species notified on the label by using Chipron LCD Array Analysis System. The results showed that 39 samples (53.4%) were labelled incorrectly. Randomly selected eleven samples were corrected by Iontek Fluorion Meat Species Identification Kit and FDS Detection System (Real Time PCR). Hence, it was found that the results obtained by DNA Microarray and Real Time PCR methods were identical (100%) with each other, and both methods should extensively be promoted for the detection of animal species in the meat and meat products.

Keywords: DNA Microarray, Animal Species Detection, Food Safety, Real Time PCR

INTRODUCTION

Protein as a macro nutrient and energy source received from meat and meat products has important building and regulatory functions in the body. It is recommended that at least 1/3 of the daily protein requirement in a well and balanced diet should be taken from foods of animal origin. Protein synthesises hormones, enzymes and immune-related species as well as it protects homeostatic balance. WHO reports that there is a positive correlation between the level in terms of development and the nutritional fact of protein rich of animal-origin in the report of the Global and
In this study, 73 samples of the meat and meat products sold in stores, meat selling markets and public bazaars located in different districts of Istanbul province were analyzed to detect the existing animal species as notified on the label by using Chipron LCD Array Analysis System; and randomly selected 11 samples were controlled for the verification of the previously found results by Iontek Fluorion Meat Species Identification Kit and FDS Detection System.

**MATERIAL and METHODS**

**Material**

In this study 33 of fermented sausages, 16 of grilled meatball, 11 of ground meat, 7 of salami and 6 of sausages sold...
in stores, meat selling markets and public bazaars located in different districts of Istanbul province were collected. All the samples were examined for notification on the label and assessment of adulteration by DNA Microarray method.

**Method**

The collected samples were placed in sterile sampling bags, and transported inside a refrigerated container kept at 4°C for sample preparation and DNA isolation. The pieces taken by means of lancet and spatula were homogenized in a blender. 0.20 gram of the homogenized sample was put into Eppendorf tubes.

DNA was extracted by following up the procedure given in Eurofins GeneScan GÈNESpin DNA Isolation Kit (Catalog no: 5224400605) as outlined in Fig. 1. The extracted samples of DNA were stored at -20°C.

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**Fig 1. DNA isolation procedure**

**Şekil 1. DNA izolasyonu prosedürü**
The extracted DNA samples were amplified by Real Time PCR (Agilent Stratagene Mx3000P) using the procedure given in Chipron LCD Array Meat Species 1.6 Kit (Chipron GmbH, Germany). Since the kit is ready to use, 12.5 µl of Chipron 2x all in one master mix, 1.5 µl of primer mix and 8 µl of sterile water were put into an Eppendorf tube, respectively. This prepared solution of 22 µl was pipetted to each of the plate wells following addition of 3 µl of DNA template. The plate was closed and was installed in Real Time PCR. Thermal processing was given as 1 cycle at 96°C for 3 min, then 30 cycles at 94°C for 30 sec, 57°C for 45 sec and 72°C for 45 sec, and finally 72°C for 3 min.

Twenty two microliter of hybridization solution and 2 µl of modulator solution were added into an Eppendorf tube. This 24 µl of mix was pipetted to each of the plate wells following the addition of 10 µl of extracted DNA samples. Chip in the kit was placed in the chip box. 30 µl of each of the plate wells was pipetted onto the lower left hand corner of each of the eight patterns (Fig. 2). Chip box was closed, allocated to standby at 35°C for 30 min, washed, dried, and then placed in the box again.

Putting the dilution solution into the Eppendorf tube 30 µl of annealing solution was pipetted into each of the patterns of the chip and allowed to standby for 5 min. After the incubation completed washing procedure was done, and chip was centrifuged for 15 sec, allowed to dry, and placed in the box again.

Thirty microliter of staining solution was put into each of the patterns of the chip, and the chip was allowed to standby for 3 min in room conditions. Following staining procedure, it was kept in washing box for 15 sec, and then centrifuged for 15 sec for drying.

**Evaluation of the Results**

Chipron LCD Array System can detect cattle, buffalo, pig, sheep, goat, horse, donkey, rabbit, hare, chicken, turkey, goose, and two duck varieties in food sample. The detection in this system is based on specific sites within 16S rRNA mitochondrial locus of all meat species in the analyzed food sample. A dark precipitate is formed by the enzyme substrate provided in the test kit, and it indicates a positive hybridization reaction. After staining procedure completed the chip was read with the scanner, and analysis was done by the software from the “Analysis-Package” provided by Chipron. Three different spots on the chip are called the control points (C) to detect a positive reaction which are located in upper-left, upper right and lower right corners, respectively. If no darker visualization occurs, the test should be repeated. The animal species was identified according to Fig. 2 and Table 1.

**Verification by FDS Detection System (Real Time PCR)**

Randomly selected 11 samples which analysed by DNA Microarray method were verified by Iontek FDS.

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**Table 1. Capture probes**

| Well No | Probe   | Specificity       | Well No | Probe   | Specificity       |
|---------|---------|-------------------|---------|---------|-------------------|
| 01      | Beef    | Bos taurus        | 08      | Rabbit  | Oryctolagus cuniculus |
| 02      | Buffalo | Bubalus bubalis   | 09      | Hare    | Lepus europaeus   |
| 03      | Pork    | Sus scrofa        | 10      | Chicken | Gallus gallus     |
| 04      | Sheep   | Ovis aries        | 11      | Turkey  | Meleagris gallopavo |
| 05      | Goat    | Capta hircus      | 12      | Goose   | Ansa albinrons    |
| 06      | Horse   | Equus caballus    | 13      | Mall. Duck | Anas platyrynchos |
| 07      | Donkey  | Equus asinus      | 14      | Musc. Duck | Cairina moschata  |
|         |         |                   |         | C       | Hyb-Cont.         |

Fig 2. Spotting points of LCD-array meat 1.6

Şekil 2. Çip noktalı ve et tür eşlemleri
Detection System (Real Time PCR) method. The DNA which previously isolated by using Eurofins GeneScan DNA Isolation Kit (Catalog No: 5224400605) stored at -20°C were used. The procedure given by Iontek Fluorion Meat Species Identification QLP 1.0 Kit (Catalog No: F0560102) was followed up. Positive and negative controls were run in duplicate whereas DNA samples were run in triple. All the solutions and materials in the kit were dissolved before use. 23 µl of PCR master mix including 12.5 µl of PCR mix, 4 µl of detection mix and 6 µl of sterile water was pipetted into each of the plate wells. Two microliter of previously extracted DNA was added onto each. The tubes were closed off tightly and placed in Iontek FDS Real Time PCR System. The thermal processing was given as one cycle at 95°C for 15 min, then 40 cycles at 95°C for 25 sec and 62°C for 20 sec. The analysis was done by the FDS software from the “Analysis-Package” provided by Iontek.

RESULTS

The results obtained by DNA Microarray indicated that 39 out of 73 samples (53.4%) were labelled incorrectly, and adulteration was made in contrary to the notifications on the label. The adulteration was detected mostly in meat balls (87.5%), ground meat (72.7%), salami (57.1%), sausages (50%) and fermented sausages (30.3%), respectively. The results are presented in Table 2. It was mostly seen that meat balls and ground meat have significantly potential risk for adulteration. Following them fermented sausage samples showed incorrect labelling with the range of 30%. On the other hand, these three types of food were having a claim of 100% beef on the labels. Hence, mostly detected meat species in meat ball, ground meat and fermented sausage samples were chicken, turkey and sheep species. No pig and equine species were detected in 79 samples. Randomly selected 11 samples out of 79 were verified by Iontek FDS Detection System (Real Time PCR). The results obtained by both of DNA Microarray and Real Time PCR were identical to each other with the range of 100%. The verified results are also given in Table 3.

DISCUSSION

The inspection of the declared composition of food stuff as notified on its label is officially an obligatory task order to protect the public benefits and health against adulteration and infectious diseases caused by zoonoses. In this study, we compared DNA Microarray (Chipron, Germany) method to Iontek FDS (Real Time PCR) System for routine use.

A variety of animal species present in the meat and meat products were examined in the past. In Turkey many studies related to the animal species detection were carried out by official authorities and academic research institutes. In Izmir province totally 116 samples of joint meat, ground meat, sausage pulp, meat ball, hamburger, canned meat, Turkish Doner, fermented sausage, salami, roasted meat and sausage were reviewed for the notifications declared on the label. The results showed that 18 samples (15.5%)
were labelled incorrectly and containing animal species other than the declared ones like horse flesh (9.5%), pork (9.5%), chicken (23.8%) in the sliced meat; pork and beef (4.8%) whereas chicken and beef mix (9.5%) in ground meat. A hundred percent of sausage pulp samples were containing chicken and beef together. It was reported that 4.8% of meat balls had pork meat, and chicken was also detected in Tas Kebap, Turkish Döner, salami, roasted grill and sausages in contrary to the notification on the label. Consequently, meat and meat products produced in Izmir were mixed with meat belonging to various animal species. Some other studies carried out in Istanbul and Bursa provinces totally 100 samples composed of 28 fermented sausages, 25 salami, 9 raw meat, 16 ground meat, 3 pastrami, 7 ham, 7 grilled meat, 5 canned meat were tested. The obtained results indicated that 11 fermented sausages (39.2%), 8 sausages (62.9%), 2 raw meat (22.2%) and 1 ground meat were contrary to the declarations on the label, and 22% of all collected samples were carrying potentially high risk for health. Another study reported that 65 of ground meat, 35 of meat ball pulp, 50 of fermented sausage pulp, 125 of fermented sausage, 75 of salami and 60 of sausage totally making 410 samples the adulteration ratio was determined to be 19.2% (79 samples). A study done in USA indicated that 62% of the meat and meat products had only one foreign species, 36% had two, and 2% had three. A similar study in the States also showed that the adulteration ratio has increased up to 46.4% In Brasil commercial samples of swine hamburgers showed no adulteration with bovine, chicken, swine or horse meats, and expectation of hamburger adulteration was not confirmed. It was found that our study and those carried out in Turkey and at abroad have delivered identical results. It is understood that the adulteration is a key tool in reducing the costing in the production of meat and meat products, preferably tried in contrary to the notifications on the label poultry, and especially encountered in processed meat products. This fact could somewhat explain the risk of zoonosis harmful to public health.

The detection of animal species in meat and meat products have been done by a variety of analytical methods. Each method has relatively advantages and disadvantages as compared to each others. Recently, Real Time PCR, a DNA based molecular technique, has been very popular in food analyses as a further step of the conventional PCR. It brings away the demand for immunological and electrophoretic methods and minimizes the risk of contamination during the testing. Real Time PCR has a sensitivity in detection of meat species by 0.1% whereas ELISA can do it less sensitive by 2%. DNA Microarray and Real Time PCR methods differentiate from each other in simultaneously detection of animal species in one reaction. The only common similarity between them is the step of DNA isolation. Microarray Analysis can enable us for detecting more than one species in one reaction only whereas Real Time PCR requires specially designed primers and probes needed to simultaneously amplify the specially selected regions of DNAs belonging to different species. This difference means longer time needed in the optimization step of primers and probes. DNA Microarray can deliver the results faster and more sensitive using amplified DNA by conventional PCR technique. Therefore, DNA Microarray method has been widely preferred for understanding mechanisms, detection of foodborne microbial pathogens and food safety, nutraceuticals and functional foods as well as following up the different expression levels of DNA in bacteria, yeasts, plants and human; genetic and mutation analyses; environmental studies; identification of antimicrobial genes, proteomics, protein-nucleic acids, protein-protein interactions, biochemical analysis of protein functions and drug development. In the recent years studies in the literature related to DNA Microarray have focused on the detection of adulteration in seafoods and meat and meat products. In our study, DNA Microarray was used to determine adulteration in some selected meat products by making verification by Real Time PCR method. It was found that both of the methods delivered the identical results. Therefore, it was seen that DNA Microarray method is fast, accurate and safe by introducing this technique firstly to Turkey for detection of foreign animal species. DNA Microarray was preferred for higher capacity of data analysis, suitability for species detection, re-usability of the results, higher analysis throughput and becoming user-friendly.

In conclusion, adulteration is a serious food safety and quality issue with an increasing prevalence in meat and meat products all over the world. Regular controls for adulteration in meat and meat products should be frequently and intensively done due to the significant increasing demand for the meat. It was found that the results obtained by DNA Microarray and Real Time PCR assays were identical with each other, and both methods should extensively be promoted for the detection of animal species in the meat and meat products.

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Detection of Animal Species in Meat Products

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