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Halal authenticity of sausage samples by qPCR analysis

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Abstract: The origin of meat products and any porcine contamination should be carefully analyzed for Halal certificaiton. Different methods are utilized for origin detection and of meat and meat products. Due to the sencitivity and selectivity, Real Time PCR is used widely in the area. In this method, even very small amounts of DNA can be determined. In this study, sausages from the most famous sausage brands in Turkey were purchased from the market and they were screened for origin detection and porcine contamination. It was found that none of the sausage samples had any porcine contamination.

Keywords: Metrology; halal food; meat product.

1. Introduction

High quality meat and meat products are expensive, therefore, adulteration of meat products such as sausages with low quality meat products increases the profit and creates a major problem in the food market. In most of the cases, pork, a cheaper alternative, is added to the sausage samples, since the texture is similar to beef meat [1,2]. However, adulteration of sausage samples with pork creates a major problem in the Islamic market, since the consumption of pork is considered haram, in addition to the health related risks [3]. Halal food market increases rapidly and they require halal food certification which require sensitive and selective measurement methods. Since sausage is blended with spices, it is very difficult to determine the origin of meat by physical properties. For the origin detection of meat and meat products, several different methods are used including DNA-based molecular detection, protein-based detection and fat-based detection [4]. DNA based methods are routinely used in several testing laboratories as it is more selective and sensitive than others.

The aim of this study is to screen the authenticity of major sausage brands in Turkish market. For this purpose, sausage samples were purchased from major brands of sausage suppliers in Turkey. Real Time PCR (qPCR), a DNA based method, was applied for origin detection and relative percentage determination.

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2. Experimental

2.1. DNA Isolation and purification

11 different sausage samples were purchased from Turkish markets (Table 1). Firstly, total DNA was extracted according to the Wizard Genomic DNA Purification Kit instruction booklet (Cat.No.A1120, Promega Corporation, USA) with slight modifications. Briefly, 50 mg of meat sample was homogenized at 230 sec/frequency in Retsch Mixer Mill MM 400 homogenizer using steel balls. Then, 600 uL of freshly prepared EDTA/0.5 M Nuclei Lysis Solution were added and vortexed to obtain a homogenous solution. After addition of 3 uL RNAse solution and 17.5 uL Proteinase K (20mg/mL), lysate was incubated at 65°C for 30 minutes. DNA was precipitated by the addition of 200 uL protein precipitation solution and the sample was centrifuged at 14.000 rpm for 5 minutes. The supernatant was transferred to another tube containing 600 uL of isopropanol and DNA was precipitated by centrifugation at 14.000 rpm for 5 minutes. The pellet was dried and dissolved in 100 µL of rehydration solution, it was incubated at 65°C for 1 h, then it was stored at -20 °C until PCR analysis. The purity of DNA extract was checked by NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA). The integrity of the total DNA was confirmed by running extracted total DNA on 1% agarose gel (data not shown).

2.2. Quantitative PCR

Amplification of extracted DNA samples was performed in duplicate with LightCycler 480 (Roche, Germany) using SYBR® SYBR Green I Master Mix. The PCR was performed in a total volume of 20 µl with 10 ng of each DNA sample and corresponding primers (Table 1, [5]). DNA primers were synthesized and purified by HPLC (Sentromer, Turkey). After an initial denaturation at 95 °C for 5 min 30 cycles were performed by denaturing at 95 °C for 15 s, annealing at 60 °C for 15 s and extending at 72 °C for 30 s. Some of the samples contained two species, for these samples, relative DNA copy numbers were calculated according to Livak et.al., 2001 [6]. The sample that contains three species, the relative concentration of each species was calculated by subtracting Ct values from 40, calculating theoretical number of DNA assuming that DNA multiplication efficiency is 2, adding them all and dividing DNA copy number of each species to the total number of DNA. Standard deviation of two data was calculated as the deviation from the mean.

3. Results and discussion

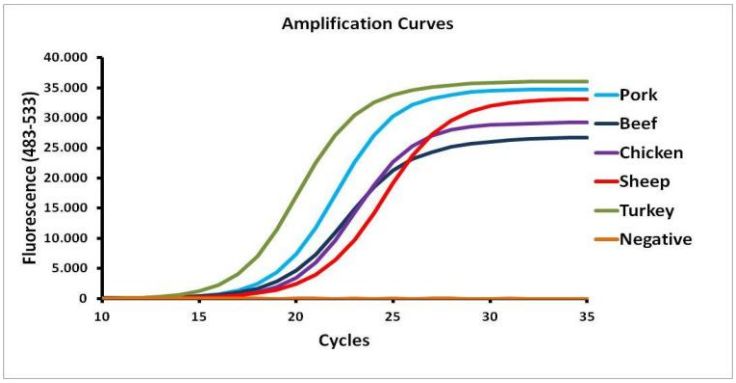
All control samples and sausage samples were subjected to DNA isolation and purification protocol. Total DNAs were successfully isolated from heat treated sausage samples. Firstly, control DNA samples (beef, sheep, turkey, chicken and pork) were analyzed with the addition of individual primers in different reaction tubes to confirm positive amplification (Table 1, Figure 1.A). Each primer was also tested with each template to test cross-reactivity between different species.

According to PCR analysis, it was found that each of positive DNA samples were amplified with corresponding primers as a single melting peak (Figure 1.B) and the size of PCR amplicons was confirmed as expected (Figure 2). Cross-reactivity was not observed between irrelevant primer and target sequences.

Table 1. Primer sequences used in PCR [5].

Meat Primers	Sequence	PCR Product Size (bp)
Beef Forward	GAACTACGGCTGAATCATCCGA	183
Beef Reverse	GGTAGGACGTATCCTATAAATGCTGTG	
Sheep Forward	ACGAGGCCTATACTATGGATCATATACC	112
Sheep Reverse	TCCTCATGGTAAAACATAGCCTATGAATG	
Turkey Forward	CCATACATTACACTGCAGACACCACT	223
Turkey Reverse	GTTGCTATGAGGGTGAGAAGTAAGAC	
Chicken Forward	AATCACGACCACCTTACAACCTTAC	72
Chicken Reverse	AAAATGTCGACCAGGGGTTTATG	
Pork Forward	ACGTAAATTACGGATGAGTTATTCGC	166
Pork Reverse	GCTGTTGCTATAACGGTAAATAGTAGGAC	

A.



B.

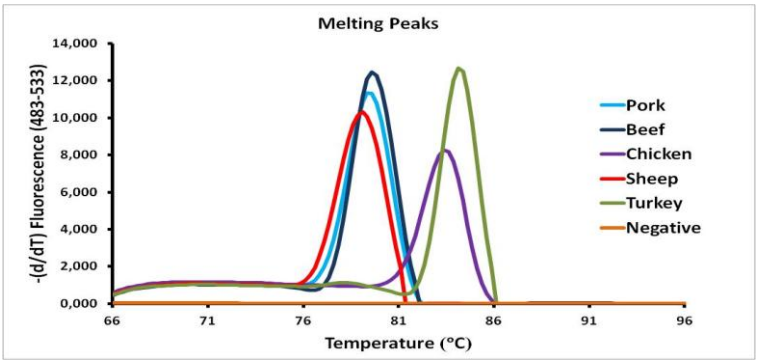


Figure 1. Real-Time PCR analysis of positive control samples. A) Amplification curve of positive control samples. B) Melting analysis of positive control samples.

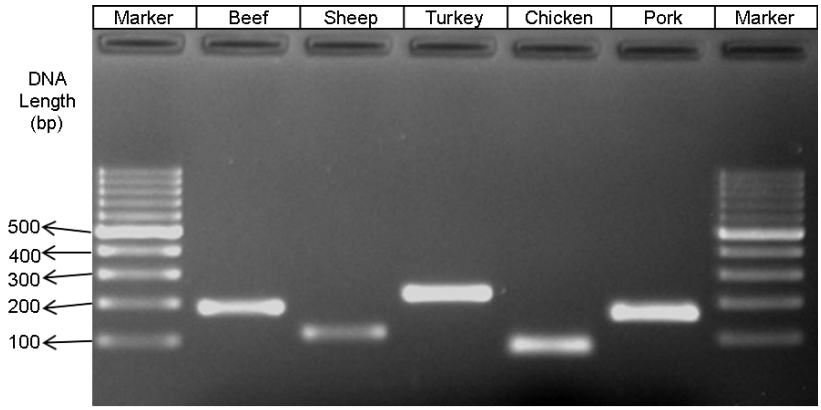


Figure 2. DNA PCR analysis of positive control samples Real-Time Analysis of positive control samples.

Secondly, all major brands of sausage samples that are sold throughout Turkey, were screened for the presence of beef, sheep, turkey, chicken and pork DNA. All samples were extracted in different tubes to avoid cross contamination. Extracted mitochondrial DNA was subjected to the amplification with all primers of beef, sheep, turkey, chicken and pork, in individual tubes. All control samples were only amplified by their corresponding primers. All beef-sausage samples were only amplified by beef primers and other primers did not result in any PCR amplification [Table 2]. Chicken and chicken/turkey sausage samples also revealed beef amplification bands on PCR. Two chicken-sausage sample contained 1,1±0,3% and 5,8±0,4% beef DNA. One chicken/turkey sausage sample contained 4,7±0,8%, 9,1±0,3% and 86,2±0,8% beef, turkey and chicken, respectively. The labeling of chicken and chicken-turkey sausages states that beef fat is included in the Sausage samples and these DNA are attributed to the presence of beef fat. None of the sausages revealed any sheep or pork DNA.

Table 2. RT-PCR analysis of sausage samples. Beef, sheep, turkey, chicken and pork mitochondrial DNA were isolated, purified and amplified with all primers in addition to the sausage samples (%)

Sample or Controls	**Beef**	**Sheep**	**Turkey**	**Chicken**	**Pork**
Beef Control	100	0	0	0	0
Sheep Control	0	0	0	0	0
Turkey Control	0	0	100	0	0
Chicken Control	0	0	0	100	0
Pork Control	0	0	0	0	100
Sausage_1, Beef	100	0	0	0	0
Sausage_2, Beef	100	0	0	0	0
Sausage_3, Beef	100	0	0	0	0
Sausage_4, Beef	100	0	0	0	0
Sausage_5, Beef	100	0	0	0	0
Sausage_6, Beef	100	0	0	0	0
Sausage_7, Beef	100	0	0	0	0
Sausage_8, Beef	100	0	0	0	0
Sausage_9, Chicken	5,8±0,4	0	0	94,2±0,4	0
Sausage_10, Chicken	1,1±0,3	0	0	98,9±0,3	0
Sausage_11, Chicken & Turkey	4,7±0,8	0	9,1±0,3	86,2±0,8	0

4. Conclusions

According to the Turkish Legislation (Republic of Turkey Ministry of Food, Agriculture and Livestock, 2012/74, No: 28488), it is not allowed to mix poultry and red meat. All the sausage brands sold in Turkey states that their products do not contain any pork or pork products. Since, minute quantities of DNA can be detected by qPCR method, this method was also utilized to screen porcine contamination, in this study. According to the results of this study, major brands of beef sausage do not contain any poultry meat and or porcine meat.

The analysis of chicken sausages revealed the presence of beef or beef products at different levels. It is allowed to use beef fat in the preparation of chicken or turkey sausages as sources of fat and it is also stated in the ingredients list of the sausages. Since DNA extraction efficiency of fat may vary considerably, these concentration differences can be explained by different DNA extraction efficiency. In a previous study, several chicken sausage and salami samples were also analyzed and it was found that beef DNA was also detected in chicken sausage and salami samples, as stated on the label of the product and none of the samples contained any porcine DNA [7].

It is important to note that, very small amounts of chicken can be found in beef sausages, this does not mean adulteration rather than the contamination. The sausages are prepared in the same production lines using the same processing machines; therefore, it is possible that minute amount of other meat/fat can be present in the sausage. However, even minute concentration of porcine products is not allowed by religion, the presence of porcine products should be accurately determined for halal certification. PCR technique is a very sensitive method in achieving detecting the smallest porcine contamination, but, since it relies on the presence of DNA, further heat processing of sausage samples may not reveal the porcine contamination which should be assessed scientifically.

According to these surveys, it is reasonable to conclude that Turkish meat market is strictly controlled by government authorities and it is safe to consume major meat products sold in Turkish market in terms of halal issue.

References

[1] K. Nakyinsige, Y. B. C. Man, A.Q. Sazili (2012). Halal authenticity issues in meat and meat products. *Meat Science.* 91, 207-214.

[2] C. Murugaiah, Z.M. Noor, M. Mastakim, L. Bilung, J. Selamat, S. Radu (2009). Meat species identification and halal authentication analysis using mitochondrial DNA. *Meat Science.* 83, 57–61.

[3] A.A. Aida, Y. B.Che Man, , C. M. V. L Wong, A. R. Raha, & R. Son (2005). Analysis of raw meats and fats of pigs using polymerase chain reaction for halal authentication. *Meat Science.* 69, 47–52.

[4] İ. Boyaci, R. Uysal, T. Temiz, E. Shendi, R. Yadegari, M. Rishkan, H. Velioglu, U. Tamer, D. Ozay H. Vural (2014). A rapid method for determination of the origin of meat and meat products based on the extracted fat spectra by using of Raman spectroscopy and chemometric method. *Eur. Food Res. Technol.* 238, 845–852.

[5] C. Camma, M.D. Domenico, F. Monaco (2002). Development and validation of fast Real-Time PCR assays for species identification in raw and cooked meat mixtures. *Food Control*. 23, 400-404.

[6] Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

[7] A. C. Gören, H. Yılmaz, S. Gündüz, B. Yalçınkaya, M. Akgöz (2013). Halal food and Metrology. *2nd. International Halal and Healthy Food Congress.* 97-107.