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DETERMINATION OF BEEF, SHEEP AND GOAT MEAT BY PCR

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Abstract. *In Azerbaijan, all traditional dishes contain meat, which is considered a sign of a healthy life. The purpose of study was the identification of meat species and the presence of cow, sheep and goat meat in the composition of sausages. For this purpose, multiplex real-time PCR kits were used. The one of baselines of the study was to observe the cross reaction between sheep and goat meat, given their similarities. Also, ussualy sausage manufacturers write on labels that the composition includes cow and sheep meat, which is very important for the Muslim population. We used the PCR kit, based on four channels reading: FAM, JOE, ROX and CY5. The results have shown, though the manufactered company not include the presence of goat meat to the labels, the goat meat has been determined. The research confirmed, that the PCR method is the one of the sensitive techniques for determination of meat species' origin.*

Keywords: identification, beef, goat, meat, PCR, sheep

Histological and classical methods were usually used to determine the identification of the origin of different types of meat. The main drawback of these methods was that the test was carried out and there were errors in the interpretation of the results (Cai, 2022). Determining the origin of meat plays an important role in food safety and consumer rights protection. Electrophoresis PCR is one of the most common methods for determining the origin of meat (Kumar, 2014; Rodr?guez, 2004, Vallejo-C?rdoba, 1998). In addition, immunological analyzes (ELISA) (Fei, 1996) and liquid chromatography (LC) are used. According to this methodology, fresh or frozen meat is tested (Ashoor, 1988).

Polymerase Chain Reaction (PCR) is a method used in molecular biology to generate multiple copies of a specific DNA template, amplicons. PCR is currently used in meat identification as the final confirmatory and accurate method (Giovannacci, 2004; Matsunaga, 1999; Vaithiyanathan, 2016).

Azerbaijan is a Muslim country and one of the most commonly eaten foods is meat. Dozens of meats and meat product producers operate in the country and note that the ingredients of the food they produce are beef and sheep. The sensitive approach of the population to this issue highlights the relevance of the topic.

The main purpose of the conducted research was to determine the presence of beef, sheep and goat meat in the sausages offered by different companies. In this way, it was possible to determine that the composition shown on the label reflects the truth.

Materials and methods. *Collection and storage of samples.* As a control group, samples of raw beef meat (n:1), lamb meat (n:1), goat meat (n:1) were selected from the market on the basis of the principle of chance. In addition, cervelat sausages belonging to the brands “Halal nemat”, “Best beef” and “Ovchular” were purchased. All samples were packed in sterile bags, each of them was marked with a corresponding identification number. Before the DNA isolation process was started, all samples were stored at -20°C.

Laboratory preparation. The molecular biological analysis room of the biosafety level 2 laboratory of the Research Institute of Veterinary Medicine was prepared for the polymerase chain reaction. To do this, the UV lamp of the biosafety level 2 cabinet was turned on for 30 minutes. The inside of the cabinet was then first cleaned with 10% bleach (chlorine) and 70% alcohol. The cabinet ventilation system was connected and the necessary consumables were collected. For this, a vortex, pipettes, nozzles, a waste container and a centrifuge were needed.

*Sample preparation***.** With regard to SOP-04 for DNA isolation: raw meat and sausages were cut into small pieces and packed in a mortar. Phosphate buffer solution was added to it at a ratio of 1/10. The tissues were then minced and homogenized. The prepared solution was poured into a sterile 15 ml tube, centrifuged at a speed of 10000 for 10 minutes, the supernatant was collected in 2 ml Eppendorf tubes and placed in a biosafety cabinet.

DNA isolation. Qiagen mini DNA kit was used for DNA extraction.

1. Add 200 µl of sample to a 1.5 ml tube.
2. 400 µl Lysis Buffer is added to it and kept at room temperature (20-24°C).
3. Add 450 µl of Binding Buffer (we check the addition of ethanol) to this and mix.

4. The mini-spin column is placed in a 2 ml Epperdorf and 600 µl of solution is added.
5. The prepared tubes are centrifuged at 14000-16000 rpm/1 min.
6. We change the tube assembled at the bottom and repeat the procedure indicated in paragraph 3.
7. Add 400 µl wash buffer 1 and centrifuge at 14000-16000 rpm for 30 seconds.
8. The mini-spin column is transferred to a new tube, 600 µl of wash buffer 2 is added and centrifuged at 14000-16000 rpm for 30 seconds.
9. Empty tube is replaced and centrifuged at 14,000–16,000 rpm for 3 minutes.
10. The filtered 1.5 ml column is placed in a tube and 50 µl of RNase-free water is added directly to the center without touching the membrane. A pause of 3 minutes is maintained at room temperature.
11. The filtered column is centrifuged for 1 minute at 14000-16000 rpm. If the process continues, then the resulting DNA (nucleic acid) is used, otherwise the sample tube is immediately placed at a temperature of -20C.

Multiplex PCR master mix. Master mix has been prepared in accordance with kit user protocol (table 1).

Table 1

Preparing the master mix

Kit Components	Quantity
Primer/Probe Mix 2xReal-time PCR Master Mix Control DNA	4 µl 10 µl X µl to
Nuclease free water	20 µl

a) The whole procedure is carried out in a biosafety cabinet.
b) Separate 0.02 ml tubes based on the number of samples and controls.
c) Components are added (Table 1)
d) Positive and negative controls are added with a volume of 5 µl.
e) Add 5 µl of nuclease-free water instead of a negative control.
Results' detection. The results were read and analysed in the FAM, JOE, ROX and CY5 fluorescent detection channels as shown in Table 2.
The prepared samples are placed in the Biorad CFX 96 PCR machine and the program cycle was runed using specialized software for PCR-maschine.

Table 2

Fluorescent detection channels

Analyte	**Fluorophore**
Beef gene	FAM
Sheep gene	JOE-HEX
Goat gene	CY5
IC	ROX

Results and discussion. The signal detected by the test is considered positive when the curve created by the fluorescence is above the threshold line (threshold). As a result, no negative amplification occurs and the lines are drawn straight. In the first analysis, beef, lamb, goat meat and cervelat sausage "Halal Nemet" were processed. As a result (Fig 1), the threshold cycle index in the Fam channel was 21.78. This shows that the beef has the gene and that the sample works. On the FAM channel, there was no lamb and goat signal, on the ROX channel 21.95, which indicates the work of internal control. The "Halal Nemet" sausage index was 20.85 on the threshold cycle, which indicates a high concentration of beef. On the HEX channel, the lamb score was 24.29 and the sample reads as working correctly. There are no indicators of beef and goat meat, the signal on the ROX channel was 10.95. The amount of lamb in the sausage was on the threshold cycle.

21.49 indicates that it contains this meat. Goat's cycle threshold on CY5 is 14.95, which is really goat. He shows a 19:45 threshold cycle rate on ROX. Other samples show a negative result, which means that the fluorescence signal does not exceed the threshold value. In the end, it becomes clear that the sausage contains the meat indicated on the label. The positive control worked in all channels and the test was valid.

In the second analysis, beef, lamb, goat meat and "Best beef" servelat sausage were processed. As a result, (Fig 2), the threshold cycle index on the Fam channel was 20.85. This shows that the beef has the

gene and that the sample works. On the FAM channel, there was no signal of lamb and goat meat, the indicator of 22.13 on the ROX channel indicates the work of the internal control. The "Best Beef" sausage index was 16.79 on the threshold cycle, which means a high concentration of beef. On the HEX channel, the lamb indicator was at the threshold cycle of 22.69 and the graph shows that the sample is working correctly. There are no indicators of beef and goat meat, the signal on the ROX channel was 30.95. The amount of lamb in the sausage is indicated at a threshold cycle of 26.38, which confirms the presence of this meat in it. However, the amount of DNA is lower than that of Halal Nemeth sausage. The threshold cycle of goat meat in the CY5 channel is 17.78, this is really goat meat. Although goat meat was not included in the sausage, the value on the threshold cycle was 24.21 and exceeded the limit on the graph. This indicates the addition of goat meat to the product. The threshold cycle indicator on the ROX channel is 24.62. Other samples show a negative result, which means that the fluorescence signal does not exceed the threshold value. The positive control worked in all channels and the test was valid.

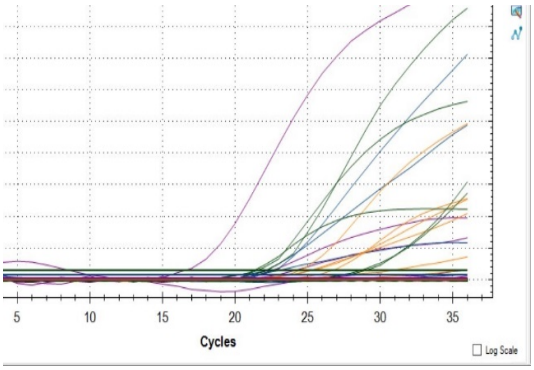

Fig.1. Sample Cycle Threshold Values (Halal Nemet)

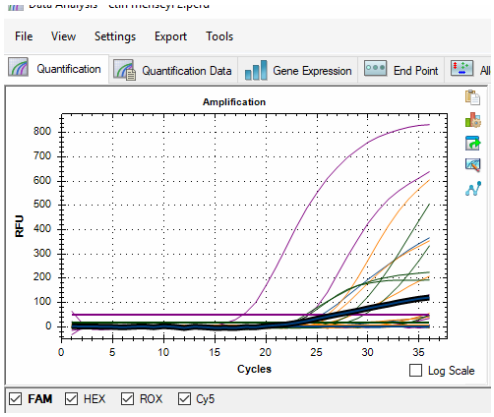

Fig.2. Sample Cycle Threshold Values (Best beef)

In the third analysis, beef, lamb, goat meat and cervelat sausage "Okhotnichya" were processed. As a result, (Fig.3), the threshold cycle index on the Fam channel was 22.57. This shows that the beef has the gene and that the sample works. There was no signal of lamb and goat meat on the FAM channel, on the ROX channel the signal indicator was 22.08, which indicates the work of the internal control. The "Okhotnichya" sausage index on the threshold cycle is 16.79, which indicates a high concentration of beef. On the HEX channel, the lamb indicator is at the threshold cycle of 23.20, and the graph shows that the sample is working correctly. There are no beef and goat indicators, the signal indicator on the ROX channel was 25.38. The amount of lamb in the sausage on the threshold cycle was 26.38, which confirms the presence of this meat in it. However, the amount of DNA is lower than in the "Okhotnichya" sausage. The threshold cycle of goat meat on the CY5 channel is 17.78, this is really goat meat. Although goat meat was not included in the sausage, the threshold cycle value was 24.21 and exceeded the limit on the graph. This indicates the addition of goat meat to the product. The threshold cycle on the ROX channel is 24.62. Other samples show a negative result, which means that the fluorescence signal does not exceed the threshold value. The positive control worked in all channels and the test was valid.

In general, the identification of different types of meat is one of the complex tasks of the supervisory authorities and is important in quality management and the issuance of the ‘Halal’ certificate. Especially in

the regions, meat products are sold in the labels of which the composition is not indicated. This not only affects the life factors of the user, but also does not respect the religious culture. Considering the high price of the tests, it is not often possible to carry out PCR diagnostics. However, DNA extraction and PCR is the most sensitive method in the identification of meat products (Rodr?guez, 2004; Zhang, 1999).

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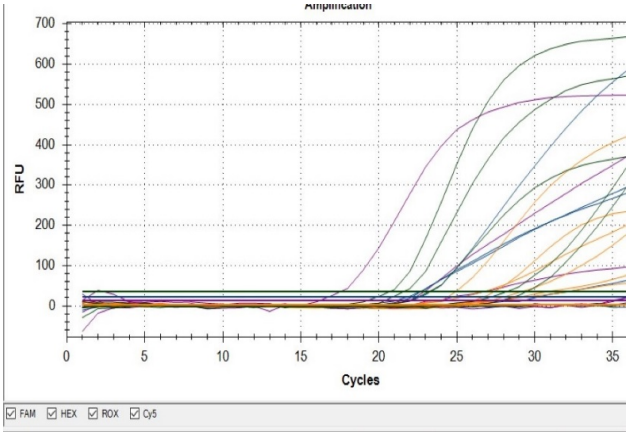

Fig.3. Sample Cycle Threshold Values (Okhotnichya)

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ВИЗНАЧЕННЯ ЯЛОВИЧИНИ, БАРАНИНИ ТА КОЗИНИ МЕТОДОМ ПЛР

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Резюме. *В Азербайджані всі традиційні страви містять м'ясо, що вважається ознакою здорового способу життя. Метою дослідження була ідентифікація сортів м'яса та наявність яловичини, овечого та козячого м'яса у складі ковбасних виробів. Для цього використовували мультиплексні набори для ПЛР у режимі реального часу. Одним із базових показників дослідження було спостереження за перехресною реакцією між овечим і козячим м'ясом, враховуючи їх генетичну подібність. Також зазвичай виробники ковбас пишуть на етикетках, що до складу входить коров'яче і овече м'ясо, що дуже важливо для мусульманського населення. Ми використовували набір ПЛР, заснований на зчитуванні чотирьох каналів: FAM, JOE, ROX і CY5. Результати показали, що компанія-виробник не вказує наявність козячого м'яса на етикетках, але козлятину було визначено. Дослідження підтвердили, що метод ПЛР є одним із чутливих методів визначення походження м'яса.*

Ключові слова: ідентифікація, яловичина, кози, м'ясо, ПЛР, вівці

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