Problems of falsification and authentication of poultry meat

Jozef Golian, Miloš Mašlej

Department of Food Hygiene and Safety
Faculty of Biotechnology and Food Sciences
Slovak University of Agriculture in Nitra
Nitra, Slovak Republic

Abstract

The monitoring of the detection kits designed to authenticate chicken and turkey meat turned out that only the first detection kit (primers and TaqMan probe designed to detect the presence of chicken meat) is sufficiently specific and sensitive, but only to 30th cycle of reactions. A reliable level detection of chicken at 100 to 0.01% in the 30th cycle of the reaction by the first detection kits, which are sufficient sensitivity for use in practice was identified.

Chicken meat, Real-Time PCR, species identification, turkey meat

Introduction

Food fraud is becoming a widespread problem and, as a result, the meat industry has begun to impose strict criteria in order to introduce effective traceability systems that would help maintain food safety and quality from farm to table (Shackell 2008). The current easy accessibility of information encourages consumers to be more attentive to the identification of meat species and the origin of the meat they eat (Kamruzzaman et al. 2012). Lifestyle aspects (vegetarianism, preference for organic foods), religion (abstaining from pork) and health problems (absence of allergens) have a fundamental impact on consumers' preferences (Ballin 2010).

With this in mind, it is necessary to ensure correct labelling as it is the only way of allowing the consumer to choose meat that satisfies his or her subjective requirements (Kamruzzaman et al. 2012). To accurately identify meat species and quantify the amount of meat in products, it is essential for sensitive and reliable analytical methods to be developed that can confirm authenticity and dispel concerns among the consumers of these products (Dooley et al. 2004).

The areas in which fraud in respect of meat and meat products is most likely to occur are:

- Meat origin (sex, age at slaughter, geographical origin)
- Meat substitution with other ingredients
- Failure to comply with declared quantities
- Type of processing treatment
  (mechanically separated meat, thawed meat, irradiation)
- Addition of non-meat ingredients (water, flour, soya protein) (Dooley et al. 2004; Ballin 2010):

The incorrectly declared composition of products on labels and the failure to declare the presence of a specific ingredient are common problems. Despite the binding nature of national and international food law regulations, it is impossible to prevent unfair business practices from harming the consumer (Ballin 2010). Although the deliberate addition of more expensive animal proteins, such as beef or mutton meat, is not generally expected, some of the possible reasons for their undisclosed presence are cross-contamination, the addition of non-conforming meat products, or rework into non-identical formulations (Cawthorn et al. 2013). A number of analytical methods are used for meat species identification which, as a rule, requires sample pre-treatment such as extraction of proteins, DNA and organic compounds (Ballin 2010). The choice of the method used depends

Address for correspondence:
Prof. Ing. Jozef Golian, Dr.
Department of Food Hygiene and Safety
Faculty of Biotechnology and Food Sciences
Slovak University of Agriculture in Nitra
Str. A. Hlinku 2, 949 01 Nitra, Slovak Republic

Phone: +421 376 414 325
E-mail: Jozef.Golian@uniag.sk
www.maso-international.cz

on the objective to be achieved and sample preparation. Not all available methods may be entirely satisfactory or sufficiently sensitive for the purpose they are meant to serve. For example, protein-based methods, which include immunological, electrophoretic and chromatographic techniques, are appropriate for meat origin identification, but not for heat-treated meat products in view of protein denaturation (Fajardo et al. 2010). The aim of this study was to find the optimum models and procedures for detecting chicken DNA by the PCR method, to determine detection sensitivity, and to highlight the advantages and disadvantages of the methodologies used.

Materials and Methods

In this study, the breast muscle tissue from the domestic chicken and the domestic turkey was analysed. The DNA extracted from chicken and turkey meat and its ten-fold dilutions were used to create calibration curves. A 100% DNA was diluted in a ten-fold series to produce 10%, 1% and 0.1% chicken and turkey meat DNA. Each dilution was homogenised by Vortex (Vortex V-1 plus BioSan, Lithuania). Two detection sets of primers designed by Jonker et al. (2008) were used. All detection kits were synthesised by General Biotech (Czech Republic). The composition of the reaction mixtures was specific, declared by the manufacturer.

Results and Discussion

The aim of this experiment was to apply the first pair of selected primers to a sample of 100% chicken meat DNA and 100% turkey meat DNA to determine the specificity of TaqMan Real-Time PCR. The first pair of primers was also used for a ten-fold serial dilution of chicken DNA (10%; 1%; 0.1%; 0.01%) to determine the detection range (sensitivity) of TaqMan Real-Time PCR. The temperature and time modes of TaqMan Real-Time PCR were set according to the recommendations of Jonker et al. (2008). The results after completed reactions in cycle 35 are shown in Plate VI, Fig. 1 and 2.

The sample of 100% chicken meat DNA crossed the detection threshold of fluorescence intensity in cycle 18.07 (Cp = 18.07). In the case of the 100% turkey meat DNA sample, the detection threshold of fluorescence intensity was crossed in cycle 31.28 (Cp = 31.28). The values at which the detection threshold of fluorescence intensity was crossed indicate that the primers used are specific up to cycle 30 of the reaction.

The detection range, i.e. the sensitivity of the reaction, can be determined from the location of individual amplification curves of specific chicken DNA dilutions (100%; 10%; 1%; 0.1%; 0.01%). Samples of individual dilutions crossed the detection thresholds of fluorescence intensity in the following cycles: 100% chicken DNA was detected in cycle 18.07 (Cp = 18.07); 10% chicken DNA in cycle 18.63 (Cp = 18.63); 1% chicken DNA in cycle 24.22 (Cp = 24.22); 0.1% chicken DNA in cycle 27.77 (Cp = 27.77); and 0.01% chicken DNA in cycle 31.31 (Cp = 31.31) (Plate VI, Fig. 1). The location of amplification curves in the chart confirms that individual ten-fold serial dilutions of the DNA were correctly prepared. The obtained values indicate that the reaction was sufficiently sensitive in view of the fact that even 0.01% dilutions of chicken samples were successfully amplified. We did not go on testing lower detection thresholds since the fluorescence signal of the 0.01% chicken sample crossed the detection threshold later than 100% turkey DNA (Cp = 31.28). The experiment was conducted in triplicate that confirmed the relevance of the results. The 100% turkey DNA was amplified in a detection range of 0.1 – 0.01% chicken DNA, and we can therefore determine the detection range of the reaction and of the first pair of primers at 0.1 – 100% chicken DNA. On the basis of the results, we evaluated the detection system tested as sufficiently sensitive and relatively sufficiently specific.

We established the linear range of the detection on the basis of the amplification curves of individual dilutions of chicken DNA that served as the calibration solutions, and plotted the calibration curve (Plate VI, Fig. 2) to quantify chicken DNA in meat products. The value of the coefficient of determination (R^2) of the linear regression with the absolute term lies within the interval <0.1>. The value of R^2 in our experiment is 0.997 and expresses

how accurately the estimated value of the trend line corresponds to the actual values. The more the value of R^2 approaches 1, the more accurate the trend line and the more successful the regression.

When testing the second detection kit designed for the authentication of turkey meat DNA, we used the same temperature and time modes as in the case of the first detection kit according to Jonker et al. (2008). On Plate VII, Fig. 3 shows the results after the completed reaction in cycle 35. The 100% turkey meat DNA crossed the detection threshold of reaction intensity in cycle 18.45 (Cp = 18.45), and the 100% chicken meat DNA in cycle 31.62 (Cp = 31.62). These values allow us to determine the specificity of the reaction which would be sufficient compared to the results of the first detection set. This experiment, however, also included ten-fold dilutions of turkey meat DNA at the 10%; 1%; 0.1% and 0.01% levels. At these levels of dilution, the detection thresholds were crossed in more than 35 cycles. We then repeated the experiment twice and found identical results. To eliminate discrepancies resulting from the dilution process, we prepared a new ten-fold dilution using newly isolated turkey meat DNA and repeated the experiment with the new dilutions. The result was almost identical. From the locations of individual amplification curves and the Cp values of 100% turkey and chicken meat DNA and individual dilutions, it is evident that 100% chicken meat DNA was amplified in the detection range of 100 – 10% of the turkey meat DNA. As we repeated the experiment using the new isolation and new ten-fold dilutions, we ruled out turkey meat DNA contamination with chicken meat DNA as the cause.

Conclusions

Detection kits intended for the authentication of chicken and turkey meat available on the market differ in their specificity and sensitivity. We found sufficient sensitivity and specificity in only one of the two detection kits used. We determined the reliable detection rate of chicken meat at the level of 100 – 0.01% up to reaction cycle 30 which is satisfactory sensitivity for use in practice.

References

Ballin NZ 2010: Authentication of meat and meat products. Meat Sci **86 (3)**: 577-587

Cawthorn D, Steinman HA, Hoffman LC 2013: A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. Food Cont **32 (2)**: 440-449

Dooley JJ, Paine KE, Garrett SD, Brown HM 2004: Detection of meat species using TaqMan real-time PCR assays. Meat Sci **68 (3)**: 431-438

Fajardo V, González I, Rojas M, García T, Martín R 2010: A review of current PCR-based methodologies for the authentication of meats from game animal species. Trends Food Sci Tech **21 (8)**: 408-421

Jonker KM, Tilburg JJ, Hagele GH, De Boer E 2008: Species identification in meat products using real-time PCR. Food Addit Contam Part A, Chem Anal Control Expo Risk Assess **25 (5)**: 527-533

Kamruzzaman, Barbin D, Elmasry G, Sun D, Allen P 2012: Potential of hyperspectral imaging and pattern recognition for categorization and authentication of red meat. Innov Food Sci Emerg Technol **16**: 316-325

Shackell GH 2008: Traceability in the meat industry – the farm to plate continuum. Int J Food Sci Tech **43 (12)**: 2134-2142

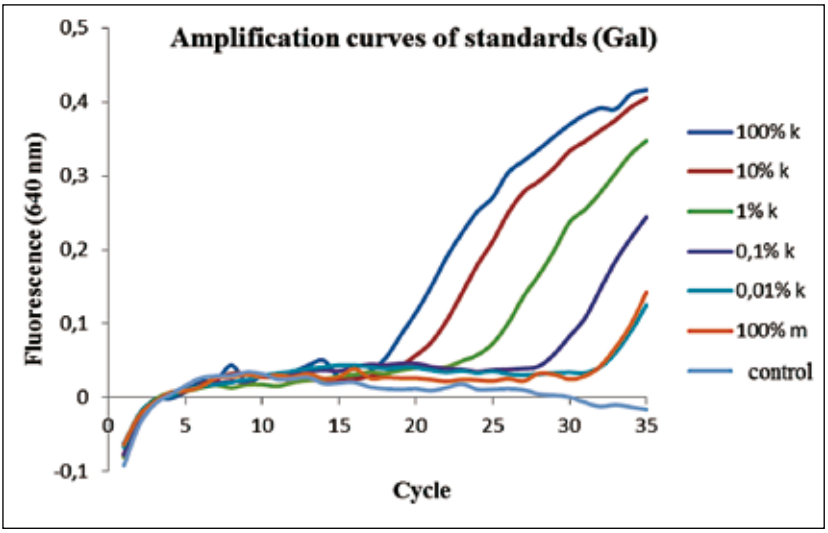

Fig. 1. Amplification curves of 100% chicken DNA, 100% turkey DNA and 10%; 1%; 0.1% and 0.01% dilutions of chicken DNA obtained with the first detection kit (Gallus F, R primers; TaqMan probe)

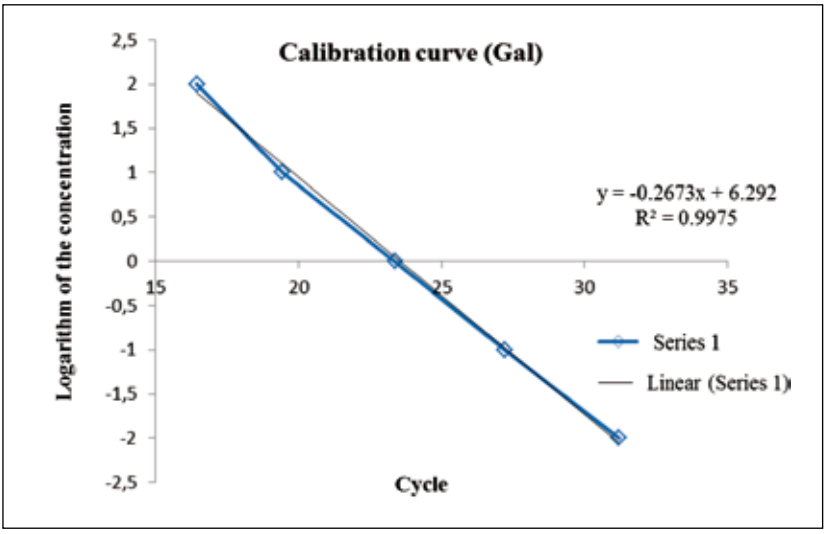

Fig. 2. Calibration curve of the first detection kit

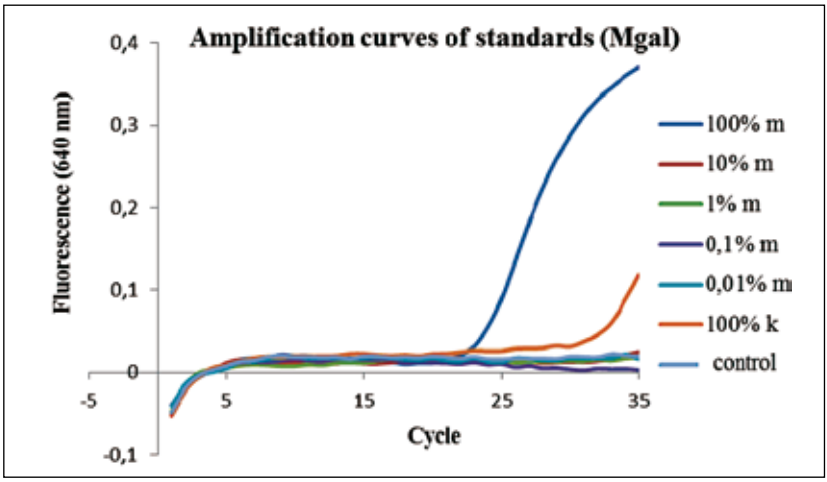

Fig. 3. Amplification curves of 100% turkey DNA, 100% chicken DNA and 10%; 1%; 0.1% and 0.01% dilutions of turkey DNA with the use of the second detection kit (Mgal F, R primers; TaqMan probe)