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DETERMINING THE PRESENCE OF CHICKEN AND TURKEY MEAT IN SELECTED MEAT PRODUCTS USING REALTIME PCR METHOD

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ABSTRACT

The one of the most convenient method for the identification of animal species in raw and processed meat products is the examination of DNA sequences. Real-Time PCR are particularly suitable because even small fragments of DNA formed during heat processing of the meat can be amplified and identified. TaqMan Real-Time PCR is a rapid, convenient and sensitive assay for meat identification. For chicken and turkey meat identification we were using species-specific primers and TaqMan probe designed on the mitochondrial cytochrome b. The intensity of the fluorescence signal has risen at a variety of different samples. We analysed sixteen the samples of turkey meat products and we found the incidence of chicken at nine samples in the range of the detection range of the reaction0.1 to 100%. Sample 8 fluorescence intensity exceeded the detection threshold in the 22.11 cycle (Cp = 22.11); Sample 6, (Cp = 23.19); Sample 1 in 27.08 cycle (Cp = 27.08); Sample 7 in 31,7 cycle (Cp = 31.7) and sample 5 in 32.32 cycle (Cp = 32.32). All Cp values for these samples fluorescence intensity exceeded the detection threshold in earlier cycles as sample the 100% turkey DNA. It follows that in the samples no. 8, 6, 1, 5, and 7 is in the range of chicken DNA detection range of the reaction, from 0.1 to 100%. Sample 11 in the cycle 27,08 (Cp = 27.08); Sample 10 in the cycle 27.8 (Cp = 27.8); sample 16 in 28.03 cycle (Cp = 28.03) and sample 13 in the cycle of 29.18 (Cp = 29.18). In recognition of the results of the monitoring of the content of chicken meat in meat products it is appropriate to further verification and testing detection kits used to work for possible use in practice since it has been found to be sufficient sensitivity and specificity to 30 cycle reaction.

Keywords: chicken; turkey; meat products; Real-Time PCR

INTRODUCTION

Meat products usually contain different kinds of meats, each of these must be declared on the label. These include hot dogs and sausages, cold meats and a variety of products containing, respectively containing pork (halal) and products with a defined share of several different kinds of meat. Prices of products vary greatly depending on the region and the current market situation **(Köppel et al., 2009)**. Authentication of meat products is currently focused mainly on demonstrating the replacement of more expensive meat cheaper, to show the presence of undeclared type of meat and use vegetable proteins because they are much cheaper compared to meat protein **(Soares et al., 2010)**. Commercial immunoassay kits are available for meat speciation, but do not normally differentiate chicken from turkey. Several electrophoretic techniques are also now available for species identification, including isoelectric focusing, sodium dodecylsulfate polyacrylamide gel, 2D and capillary zone electrophoresis. These techniques are, however, not reliable for resolving mixtures of meat species, or identifying species in highly processed meat products. The protein profiles of a single species produces a complex banding pattern, and even small amounts of protein from other species will often overlap the species-specific bands making interpretation of the resulting profile equivocal. This is compounded by the problem that heat treatments denature proteins, destroying the profile of water-soluble proteins **(Hird et al., 2003)**.

Counterfeiting of meat and supervision of food has their roots in the distant past. Since ancient times, some people are willing to adulteration of food illegally enriched at the expense of financial loss and other health risk. Each company felt the need for independent oversight over the quality and wholesomeness of food, to protect consumer-focused attention of civil, social and civil authorities. Food adulteration remains a serious problem and present **(Obrovská et al., 2002)** The Slovak Republic is a need for verification of genuineness of certain products as a necessary part of a comprehensive investigations on the quality of the goods in terms of consumer protection, together with the fight against counterfeit products in the package itself or directly for sales **(Takáčová, Bugarsky et al., 2010)**. To detect the type of meat in the composite sample were discovered many different methods, for example high performance liquid chromatography **(Espinoza et al., 1996)**, electrophoresis **(Ozgen-Arun, Ugur, 2000)** and enzyme analysis **(Hajmeer et al., 2003)**. One of the most specific methods for detecting food adulteration PCR is a method allowing precise identification of materials of biological origin **(Lepešková, 2002)**.

The aim of this work is to evaluate the determination of the presence of chicken and turkey meat in selected meat products using Real-Time PCR.

MATERIAL AND METHODOLOGY

We analysed 16 different meat products specified percentage of turkey meat purchased on the Slovak market (Tab 1). DNA were isolated by phenol - chloroform extraction, preceded skiing individual samples (sample size was 1 mm) in 600 ml of lysis solution with the addition of 20 ml of the enzyme proteinase K. TaqMan Real-time PCR was carried out in the capillary reaction cycler LightCycler® 1.5 (Roche) and the results were evaluated with the help of the LightCycler software version 4.5 (Roche, Germany), which during the PCR reaction automatically creates a graph of the fluorescence intensity of the number of cycles.

Sets of primers and TaqMan probes were designed according to **Jonker et al. (2008)** and all primers were synthesized by General Biotech (Czech Republic).

Designed primers were derived from the sequences of a specific gene cyt b. The sequence of the primers and TaqMan probes of the first and second sets of detection are listed in Table 2.

The individual primers and TaqMan probes were supplied in lyophilized form. Dissolving the freeze-dried in ultrapure water (Milli-Q H_2O) were obtained 10x concentrated stock solutions of primers, which were stored at -20 °C. Primers from stock solutions were diluted working solutions so that their final concentration of 10 pmol.μl^{-1}. Working solutions were stored at 2 - 8 °C. Lyophilized TaqMan probe from first and second detection kit was dissolved in ultrapure H_2O directly to a working concentration of 5 pmol.μl^{-1}. In a reaction mixture, we used the components necessary for optimum progress of the reaction: Colorless GoTaq® reaction buffer, $MgCl_2$, dNTP mix, individual primers and probes, and a dye ROX GoTaq® Hot Start Polymerase. We used GoTaq® Hot Start polymerase having polymerase activity blocked. Restoring polymerase activity occurs at initiation

Table 1 Analyzed meat products with percentage content of turkey meat.

no.	Product	Type and % of the declared meat content
1.	*Admirál turkey ham*	Turkey breast 64 %
2.	*Turkey breast ham, exclusive*	Turkey breast 90 %
3.	*Turkey ham*	Turkey breast 88 %
4.	*Turkey ham for children*	Turkey breast 83 %
5.	*Milled turkey meat product*	Turkey breast 92 %
6.	*Turkey breast ham*	Turkey breast 80 %
7.	*Mortadella with turkey meat*	Turkey breast 45 %
8.	*Turkey ham*	Turkey breast 71 %
9.	*Premiér ham specialty*	Turkey breast 63 %
10.	*Turkey ham*	Turkey breast 83 %
11.	*Admirál turkey ham*	Turkey breast 64 %
12.	*Turkey ham*	Turkey breast 83 %
13.	*Admirál turkey ham*	Turkey breast 64 %
14.	*Turkey ham*	Turkey breast 88 %
15.	*Turkey ham for children*	Turkey breast 83 %
16.	*Turkey breast ham*	Turkey breast 80 %

Table 2 Sequence of primers **(Jonker et al., 2008)**.

Primer	Bp	Sequence
Gallus F	27	5´-TCTCACTTACACTACTTGCCACATCTT-3´
Gallus R	23	5´-CGTGTGTGTCCTGTTTGGACTAG-3´
Gallus P	27	5´-(FAM) –CACTGCAACCTACAGCCTCCGCATAAC-(BHQ)-3

denaturation at 94 - 95 °C for 2 minutes. This system eliminates nonspecific amplification and creating primer - dimer. Mastermix is added to the reference dye ROX, which is used for normalization of the reporter signal. The normalization of the signal is essential for the prevention of signal variations caused by the construction of the device frequently. Preparation of the reaction mixture was carried out in the UV-cleaner box (BioSan, Lithuania). Capillaries are adapted to the volume of the reaction mixture from 10 to 40 ml. After adding the desired amount of DNA we conclude capillaries and quickly spun on a centrifuge. After inserting the capillary into the rotary plate of thermo cycler (LightCycler 1.5) we recorded the intensity of the fluorescent signal after each cycle measured at a wavelength of 640 nm.

RESULTS AND DISCUSSION

We determined the incidence of chicken meat in 16 selected product with the declared percentage of turkey meat (45 - 92%) without such additives of chicken meat.

In Figure 1, we can follow the fluorescence signal of DNA product of samples 1-8 and 100% chicken and 100% turkey DNA. The intensity of the fluorescence signal has risen at a variety of different samples. Sample 8 fluorescence intensity exceeded the detection threshold in the 22.11 cycle (Cp = 22.11); Sample 6, (Cp = 23.19); Sample 1 in 27.08 cycle (Cp = 27.08); Sample 7 in 31,7 cycle (Cp = 31.7) and sample 5 in 32.32 cycle (Cp = 32.32). All Cp values for these samples fluorescence intensity exceeded the detection threshold in earlier cycles as sample the 100% turkey DNA

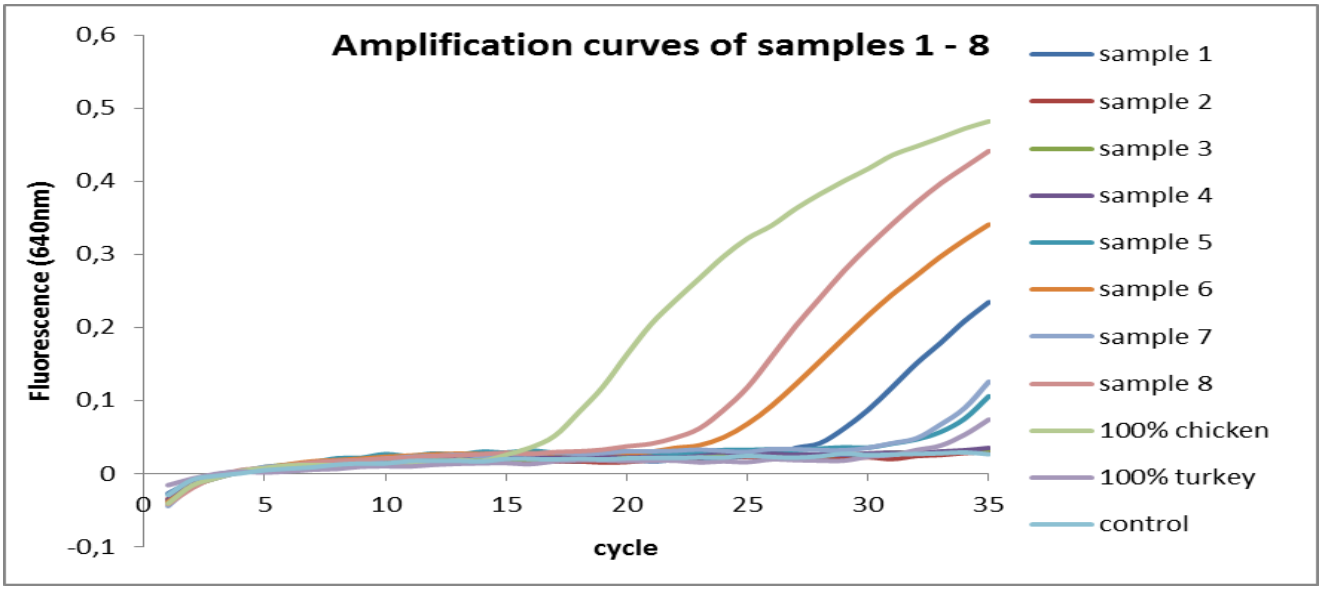

Figure 1 Amplification curves of samples 1 – 8.

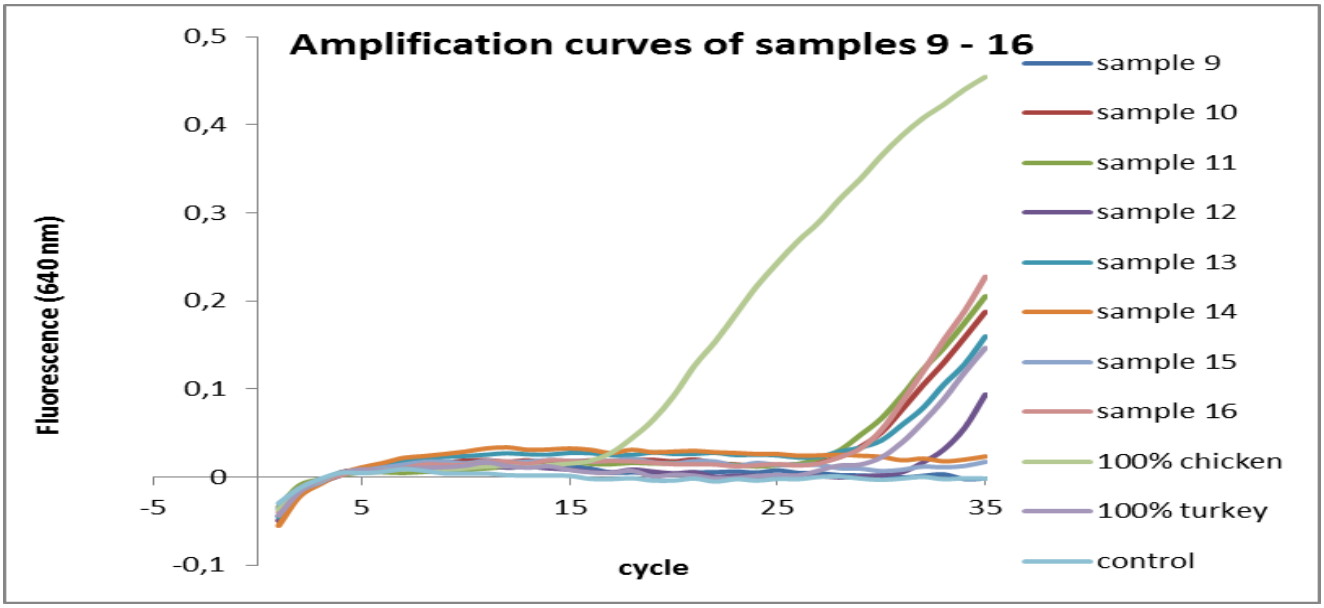

Figure 2 Amplification curves of samples 9 – 16.

(Cp = 32.94). It follows that in the samples no. 8, 6, 1, 5, and 7 is in the range of chicken DNA detection range of the reaction, from 0.1 to 100%. In samples 8, 6, 1 we observed a high incidence of chicken, which is not listed on the label in the form of impurities. With a decreasing concentration of chicken in the sample increases the number of cycles required to detect the DNA. Samples 7 and 5 are close to the lower limit of detection reactions such extent. Samples 2, 3 and 4, the fluorescence intensity exceeded the detection threshold for more than 35 cycles. For these samples, we can exclude the presence of chicken in connection with counterfeiting turkey meat on the packaging. Firgure 2 shows the amplification curves of the samples 9 - 16, the 100% chicken and 100% turkey DNA. The fluorescence intensity in the four samples exceeded the detection threshold has been exceeded rather than at the 100% turkey DNA. Sample 11 in the cycle 27,08 (Cp = 27.08); Sample 10 in the cycle 27.8 (Cp = 27.8); sample 16 in 28.03 cycle (Cp = 28.03) and sample 13 in the cycle of 29.18 (Cp = 29.18). Cp value at 100% strength turkey DNA was 29, 24 Fluorescence intensity when the sample 12 exceeded the detection threshold in the cycle 31,38. If we consider that we have established a detection range of up to 30 cycles of the reaction and sample 12 is out of the detection range of the reaction. The fluorescence intensity of the samples 9, 14 and 15 exceeded the detection threshold for more than 35 cycles, hence are also located outside the detection range of the reaction.

Using primers designed to identify chicken DNA, we were detected in chicken DNA unknown samples representative products of the stated percentage of turkey meat (45 - 92%) without the additives of chicken, in the four samples in the range of 0.1 - 1 %. The fluorescence intensity in the two samples exceeded the detection threshold for 30 cycles of reaction and therefore for these samples we cannot confirm the presence of chicken. A lower Cp value means a larger amount of initial target DNA **(Laube et al., 2006).** In the study of **Cheng et al. (2014)** succeeded in blood products successfully detected 1% strength addition of various types of blood. **You et al. (2014)** using a detection system based on cytochrome b to identify the 2% share of chicken. In studies of **Cheng et al. (2014)** and **You et al. (2014)** cross-reactivity was observed. **López-Andreo et al. (2005**) reported the detection of DNA in excess of 10%, efficiency of detection of 5 - 10% content was reduced to below 80% and 5% were able to detect species but was not effectively quantified.

CONCLUSION

Real-time PCR is a technique particularly suitable for its ability amplification and identification of small fragments of DNA resulting from thermal treatment of meat. The fluorescence intensity is measured directly during the reaction, which reduces the number of operations needed to evaluate the samples and also the possibility of contamination of the sample. Is a molecular method that can quantify the amount of the DNA. Comparing data from unknown samples with standard samples, it is possible to determine the meat content of the sample. We analysed sixteen samples of turkey meat products and we found the incidence of chicken at nine samples in the range of the detection range of the reaction 0.1 to 100%. It is appropriate to further verification and testing detection kits used to work for possible use in practice since it has been found to be sufficient sensitivity and specificity to 30 cycle reaction.

REFERENCES

Espinoza, E. O., Kirms, M. A., Filipek, M. S. 1996. Identification and quantification of source from hemoglobin of blood and blood mixtures by high performance liquid chromatography. *Journal of Forensic Science*. vol. 41, p. 804-811. [cit. 2014-07-15] Available at: http://www.fws.gov/lab/pdfs/espinoza_etal.1996.pdf

Hajmeer, M., Cliver, D. O., Provost, R. 2003. Spinal cord tissue detection in comminuted beef: comparison of two immunological methods. *Meat Science*. vol. 65, no. 2, p. 757-763. http://dx.doi.org/10.1016/S0309-1740(02)00278-4 PMid:22063437

Hird, H., Goodier, R., Hill, M. 2003. Rapid detection of chicken and turkey in heated meat products using the polymerase chain reaction followed by amplicon visualisation with vistra green. *Meat Science*. vol. 65, no. 3, p. 1117-1123. http://dx.doi.org/10.1016/S0309-1740(02)00341-8 PMid:22063694

Cheng, X., He, W., Huang, F., Huang, M., Zhou, G. 2014. Multiplex real-time PCR for the identification and quantification of DNA from duck, pig and chicken in Chinese blood curds. *Food Research International*, vol. 60, p. 30-37. http://dx.doi.org/10.1016/j.foodres.2014.01.047

Jonker, K. M., Tilburg, J. J., Hagele, G. H., De Boer, E. 2008. Species identification in meat products using real-time PCR. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess*. vol. 25, no. 5, p. 527-533. http://dx.doi.org/10.1080/02652030701584041 PMid:18473208

Köppel, R., Zimmerli, F., Breitenmoser, A. 2009. Heptaplex real-time PCR for the identification and quantification of DNA from beef, pork, chicken, turkey, horse meat, sheep (mutton) and goat. *European Food Research and Technology*, vol. 230, no. 1, p. 125-133. http://dx.doi.org/10.1007/s00217-009-1154-5

Laube, I., Zagon, J., Brpll, H. 2007. Quantitative determination of commercially relevant species in foods by real-time PCR. *International Journal of Food Science & Technology*, vol. 42, no. 3, p. 336-341. http://dx.doi.org/10.1111/j.1365-2621.2006.01249.x

Lepešková, I. 2002. Využití molekulárních metod při druhové identifikaci masa. *Maso*. vol. 5, p. 27-28.

López, I., Pardo, M. A. 2005. Application of relative quantification Taqman real-time polymerase chain reaction technology for the identification and quantification of *Thunnus alalunga* and *Thunnus albacares*. *Journal of Agricultural and Food Chemistry*. vol. 53, p. 4554-4560. PMid:15913324

Obrovská, I., Steinhauserová, M., Nemola, M., Krkoška, L. 2002. Identifikace druhu masa v masných výrobcích. *Veterinářství.*, vol. 52, 2002, p. 421-423. ISSN 0506-8231.

Özgen-Arun, O., Ugur, M. 2000. Animal species determination in sausages using an SDS-PAGE technique. *Archiv für Lebensmittel Hygiene*. vol. 51, p. 49-53. ISSN 0003-925X.

Soares, S., Amaral, J. S., Mafra, I., Oliveira, M. B. P. P. 2010. Quantitative detection of poultry meat adulteration with pork by a duplex PCR assay. *Meat Science*, vol. 85, no. 3,

p. 531-536. http://dx.doi.org/10.1016/j.meatsci.2010.03.001 PMid:20416827

Takáčová, D., Bugarský, A. 2010. *Súdne veterinárske lekárstvo a prípady znaleckého dokazovania.* Vysokoškolská učebnica. Košice : UVLF, p. 425. ISBN 978-80-8077-201-7

You, J., Huang, L., Zhuang, J., Mou, Z. 2014. Species-specific Multiplex Real-time PCR Assay for Identification of Deer and Common Domestic Animals. *Food Science and Biotechnology*, vol. 23, no. 1, p. 133-139. http://dx.doi.org/10.1007/s10068-014-0018-3

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