Detection of counterfeit beef using real-time PCR

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Abstract

In recent years, along with the growing purchasing power of the population, there has been an increasing demand for quality meat products in which the dominant component should be only meat. Meat, in a broad sense, is defined as any edible animal part, including blood. However, if one wants to buy a quality meat product, he or she expects that the dominant component should be meat and not its various substitutes. In practice, we usually have to deal with meat products that contain very small amounts of meat or even none at all (cheap sausages made from skin and mechanically separated meat). For laboratory analysis, we used calibration samples containing from 100% to 0.1% of beef, as well as finished meat products such as ordinary sausages (20% beef), ordinary sausages (10% beef), fat sausages (7% bovine meat) and Liptovská salami (10% beef). In each of the experiments, the goal was to quantify the beef content using TaqMan real-time PCR assays: the calibration curve method should always clearly demonstrate the relationship to a change in a sample containing beef, which is not true for samples of finished meat products. When quantifying the content of beef in meat products, it is necessary to know all of the ingredients in its manufacture in order to take into account the presence of substances of increasing bovine DNA content.

Beef, Real-Time PCR

Introduction

Counterfeiting meat products as a modern day trend

The word counterfeit comes from the Latin word "*contrafactio"*, and means a fraudulent imitation. Modern consumers are requiring more accurate information about the composition of their food. This is connected to modern trends, lifestyle (vegetarianism), various types of healthy diets, religious traditions, the possible threat from genetically modified food, the increasing incidence of various allergies and the consumption of traditional or exclusive specialties. In such cases, food producers are tempted by economic reasons to substitute more expensive components with cheaper ones. It is primarily expensive products or products that are produced in large quantities that are counterfeited – due to higher profits (Lees 2003; Peris and Gilbet 2009).

In most cases, the consumers themselves are unable to reliably recognize counterfeit food, which is something food manufacturers are counting on. Only in some food products is it possible to detect such fraudulent imitations through the sensory properties of the food. It is not possible to clearly prove whether a product is counterfeit or original without a comprehensive assessment of the food samples in laboratories using a variety of methods such as PCR, magnetic resonance, chromatography, microscopy, and enzyme analysis. Such monitoring ought to be among the priorities of inspection authorities (Koukal 2010).

Selected methods suitable for the detection of meat in meat products

In the case of the analyses of protein in meat products, it is necessary to consider what goes into the product as raw materials, then choose a method for the determination of analytes,

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verify it, validate it and then put it into practice. This was carried out in accordance with appropriate STN and ISO standards for meat and meat products for the determination of the total content of protein, water, fat, ash, soya, milk proteins or other sources of nitrogen (Fašiangová 2006).

Most meat products are made up of two or more types of meat. Whereas each type of meat is specific and has its own DNA, using multiplex real-time it is possible to determine the quantities and types of meat used in the manufacture of meat products. It has been proven that this method is applicable, precise and accurate when testing the various meat products offered in shops. This method allows for a variety of inspections to be carried out on the composition of meat products. Pork, beef, chicken and turkey are the most widely used raw materials in the manufacture of meat products in Europe. The price of meat differs from one type to another, which can lead businessmen to substitute meat products. Primarily, the declared (more costly) kind of meat may be exchanged for another (cheaper) one. In the worst case, this may lead to the complete substitution of meat using a variety of additives. It is therefore necessary to establish a method that will allow us to quickly and very accurately determine the composition of all meat products. The multiplex real-time is definitely such a method (Köppel et al. 2008).

The basis of a successful reaction is the use of intact DNA segments that are to be amplified (replicated). A very important prerequisite for a successful reaction is the design of suitable primers to ensure the specificity of the reaction. The design of oligonucleotide primers and the programming of reaction steps are based on the general knowledge of DNA structure and on the knowledge of the sequence to which the corresponding oligonucleotides are complementary. It must therefore be emphasized that for PCR it is necessary to know the sequences of at least the border stretches of the fragment to be amplified (Králová et al. 2007).

PCR is a molecular biological method for the detection of specific DNA markers with a high degree of sensitivity. This method is used in the monitoring of food hygiene, just as it is in the area of analyzing microbial contaminants and allergens. The methods consist of a one-or two-stage culture growth, cell lysis with possible rapid purification of the preparation, and of the analysis of specific sequences of DNA using PCR with the continuous fluorometry (real-time PCR). The developed methods have very good analytical parameters (inclusivity, exclusivity, detection limit), are generally equivalent to classical microbiological methods, and are more favorably compared to them – in particular by providing results usually on the following day after sampling (Kuchta 2009).

Materials and Methods

We used samples from a range of different types of raw beef and poultry (vacuum packed). We then isolated DNA from the samples using NucleoSpin®Food Isolation Kit (Macherey-Nagel) for genomic DNA isolation from the food.

For the analysis, we used isolates of DNA diluted by decimal dilution in order to obtain 100%, 10%, 1%, and 0.1% bovine DNA (Table 1).

The set of primers and TaqMan probes used were designed according to REA et.al. (2001) and all of the primers were synthesized by the MWG-Biotech AG company. Primers derived from sequences of a specific cyt-b gene were designed for the detection of the given types of meat (Table 2).

TheTaqMan real-time PCR method together with the LightCycler®TaqMan®Master (Roche) kit were used for the analysis. The PCR reaction was carried out in capillary-based LightCycler®1.5 (Roche) with the help of LightCycler Software 4.05 (Roche).

Table 1. Preparation of samples for analysis

Samples	Preparation	Concentration of bovine DNA
sample 1	1µl 100 % Bovine + 9 µl 100 % Poultry	10%
sample 2	1 µl 10% Bovine + 9 µl 100% Poultry	1%
sample 3	1 µl 1% Bovine + 9 µl 100% Poultry	0.10%
sample 4	100% Bovine	100%

Table 2. Sequence of primers

Por. No.	Identification	Sequence
1	CONP - F-2	5′-CTT CTT ATT CGC ATA CGC AAT CTT ACG ATC-3′
2	BOVP-R-2	5′-TGG AGG TGT GTA GTA GGG GGA TTA GAG CA-3′
3	BUFP-R-2	5′-GGC ATT GGC TGA ATG GCC GGA ACA TCA TA-3′

Table 3. Course of PCR reaction

Steps of PCR cycle				
Initial denaturation	Denaturation	Hybridization	Elongation	Final elongation
95 °C, 10 min.	95 °C, 10 s	63 °C, 30 s	72 °C, 2 s	40 °C, 30 s
45 repeat cycles				

Results and discussion

Determination of the detection sensitivity of the TaqMan real-time PCR method

To achieve our objective, we proposed the following 3 different concentrations of samples: 100%, 10%, and 1% concentrations of bovine DNA in the samples. With all of the analyzed samples it was possible to correctly determine the cycle in which the fluorescence intensity exceeded the level of the non-specific background. After isolating the DNA from the samples, we were able to obtain a RealTime PCR profile (shown in Figure 1), which clearly shows that as the concentration of impurities in a sample of beef decreases, the number of cycles to achieve the limit fluorescence increases, as does the transition curve of fluorescence to the linear growth phase. From the figure it can also be seen that the produced fluorescence curves have a clearly demonstrated relationship to the changing beef content in the sample.

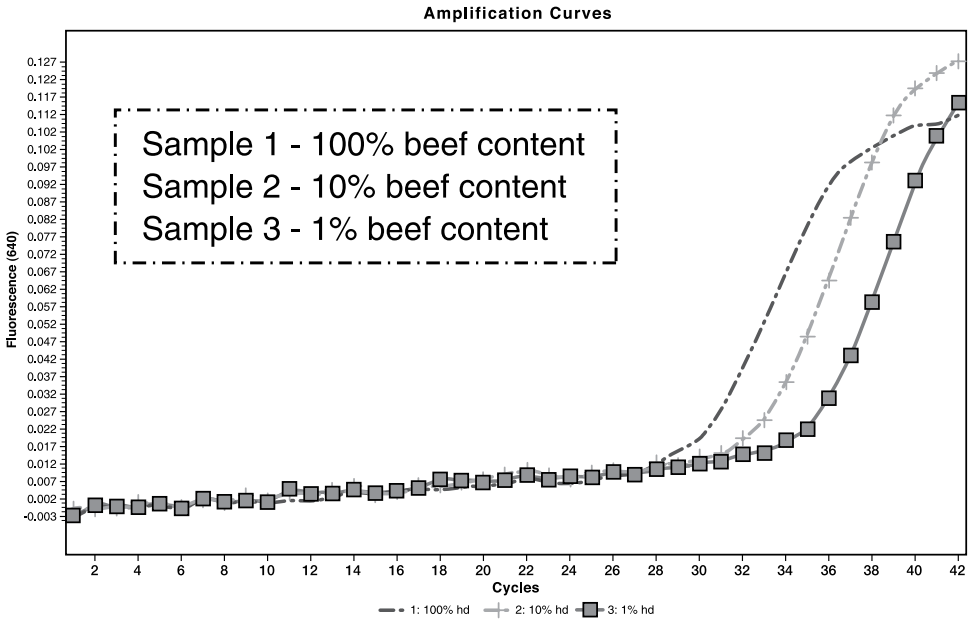

Fig. 1. Fluorescence curves of samples with 100%, 10% and 1% beef content

In their research, Wolfe et al. (2004) were engaged in the quantification of beef in sausages using various DNA methods. Their results have shown that the TaqMan real-time PCR method is the most appropriate in determining the beef content of meat products.

Conclusions

We recommend the TaqMan PCR method for the determination of the presence of beef in meat products, which would thereby lead to the exposure of possible counterfeiting: when the packaging of a meat product states that it contains beef but in fact does not include any. The limit of detection that we described was set at a level of 0.1% of beef content in direct correlation between the concentration of meat from beef cattle in a sample and the recorded intensity of fluorescence and the curve of its growth.

In addition, through this analysis we have confirmed the precision and accuracy of the preparation of calibration samples with 100%, 10% and 1% beef content, as well as eliminated the possibility of contamination of the samples during their preparation.

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