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REVIEW OF NEW TECHNOLOGIES USED FOR MEAT IDENTIFICATION

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Abstract

The present article represents an analysis of trends in development of test-systems for identification of meat. These test systems are commonly used in food production and research laboratories. The relevance of development of methods for identification of meat kinds is related not only to the food restrictions, which are practiced in some religions and related to consumption of certain types of meat, but also with the hygienic aspects of food production. Also, this research is inspired also by the acute issue of food products adulteration and the replacement of one type of meat with another one. The article considers the trends in the development of microanalysis method that use immunochromatic research, i. e. methods based on molecular biology. Also this article considers the devices that do not use chromatographic methods of analysis. Examples of the development of test systems based on various methods of analysis for the identification of meat are given below. Attention is focused on the prospects of combining these methods, including colorimetric methods for identification of meat. It is also specified that the emergence of new dyes and new enzyme systems, suitable for use in enzyme-immunoassay, can enhance the sensitivity of these test systems. It is also noted that the development of technologies associated with sorbents can contribute to a better separation of the test substrates and this way to increase the sensitivity of the test in case of small amounts of test substrate. It is also noted that the use of various types of isothermal amplification can reduce the analysis time necessary for meat identification. Various schemes of devices for microanalysis are given; their advantages and disadvantages are listed. An example of proteomes application for meat identification is given. It is shown that this method can also be applied in the heat treatment of meat. The prospects for the development of such devices are analyzed. It is concluded that the development of systems for microanalysis in the form of quick tests is quite relevant and promising. It is indicated that theoretically in the future such analytical systems, due to the use of microfluidic technologies, will be able to combine several methods. The authors proposed to use machine-aided cognition methods to analyze data obtained from similar test systems in order to increase their sensitivity.

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Introduction

Meat identification is necessary to determine the fact of substitution of one type of meat for another. In some cases, contamination with a foreign type of meat does not occur purposefully during the production process. At the same time, adulteration of semi-finished meat products is widespread both in developed and developing countries [1–7]. One of the important reasons for the relevance of meat identification is certain religious prohibition on consumption of certain types of meat. Currently, identification methods based on the analysis of proteins and genetic sequences are widely used [3,6,8,9,10,11]. These methods include analysis which uses gel electrophoresis, isoelectric focusing, chromatography, and enzyme immunoassay [12–15]. Other methods used for meat identification include PCR (polymerase chain reaction), sequencing, and various types of DNA hybridization [16]. These methods are based on determination of specific nucleic acid sequences, peculiar for the sought-for type of meat. As a rule, during the process of DNA analysis, amplification of certain DNA sectors occurs, which are further investigated [9]. There are also methods that allow amplifying DNA at a constant temperature, without any cyclic change in temperature, peculiar for PCR, for example, such methods include loop-mediated isothermal amplification (LAMP) [8,17]. Other similar methods are cross-priming amplification (CPA), recombinase polymerase amplification (RPA) and SEA amplification (Denaturation Bubble-mediated Strand Exchange Amplification) [18,19,20].

The devices used for quick identification of meat are of particular interest. These test systems and devices are in demand in food production [1].

Systems of microanalysis

Meat analysis systems with compact dimensions are called microanalysis systems. According to the principle of their operation, they are divided into two types. The first type of devices is aimed to study of the DNA sequence [18]. The second type of devices runs one of the variations of enzyme immunoassay or immunofluorescent analysis [12,13].

Most often they are executed in the form of test strips, i. e. in such a system the sorbent is applied directly on the test-strip, or the strip itself consists of a material that has the sorbent properties used for chromatography. In some cases, before the analysis a certain preparation of the sample is required, for example, extraction of DNA [21]. Some test systems combine both methods based on the analysis of DNA sequences and immunofluorescence analysis.

Immunochromatographic analysis

Immunochromatographic analysis implements the mechanisms of thin layer chromatography. The principle of this system is as follows: when a liquid sample is applied on the test strip, the dissolved components migrate along the sorbent and separate due to chromatographic mechanisms. To visualize the substances separated on the chromatographic system, the antibodies are used, labeled with a dye. In this case antibodies can bind in a direct or competitive manner. The direct method implies that the test substrate migrates along the chromatographic strip and binds to antibodies thus forming a sandwich-like structure. A competitive method is implemented when it is necessary to detect low molecular weight compounds. In this case, a system is implemented not only when the analyzed compound is there, but also its analogue is there too. The test compound and its analogue compete for binding with a limited number of specific binding centers located on antibodies.

Usually a strip of thin layer chromatography visualizes two zones arranged as a line, called a test line (T-line) and a control line (C-line). As a sorbent in such test systems they use a nitrocellulose-based material, it is the most popular. However, there are studies demonstrating the possibility of using other materials for immunochromatographic analysis [22,23]. The main problems in creating such test systems are diffusion and loss of selectivity [1, 24]. It is also noted that when developing such a test system, the processes of obtaining, labeling, amplification, and modification are simpler with aptamers compared with antibodies [1].

One of the advantages of portable test systems based on immunochromatographic analysis is the ability to detect test results visually, without the use of special equipment. It is reported that in some cases, an increase in the level of the fluorescent signal from the test and control lines increases the sensitivity of the test system [1,25,26,27]. Nardo et al. developed a two-color (red and blue) detection test system, but with one test line (T-line) [28]. A combination of both immunochromatographic analysis and surface Raman spectrometry (*SERS, Surface enhanced Raman spectroscopy*) has been reported [29,30]. Fu et al. combined the SERS method with a competitive immunochromatographic assay and found that the system was about three orders of magnitude (the authors probably meant three times) more sensitive than a similar commercially available kit [31]. Wang et al. reported that the portable immunochromatographic system in combination with the SERS method is 10,000 times more sensitive in comparison with the aggregation-based colorimetric method [32]. The sensitivity of the test system can also be increased by optimizing of the mathematical methods in data processing. Thus, the application of approaches using artificial neural networks or other machine-aided cognition methods is relevant and promising.

Methods of molecular biology used to identify meat

One of the common methods of molecular biology is PCR. This method is known for a long time. It serves as the basis for the methods like sequencing and various types of isothermal reactions. The use of the latter methods in diagnostic systems is particularly promising. Most methods, based on PCR or some type of isothermal amplification, require sample preparation immediately before the analysis (homogenization, nucleic acid isolation). Some test systems use identification of mitochondrial DNA sequence [19,33,34]. So Zhao et al. developed a test system for turkey meat identification in food products [34]. This diagnostic system showed no cross-reactivity with any of 21 other animal meats and plant species (Figure 1).

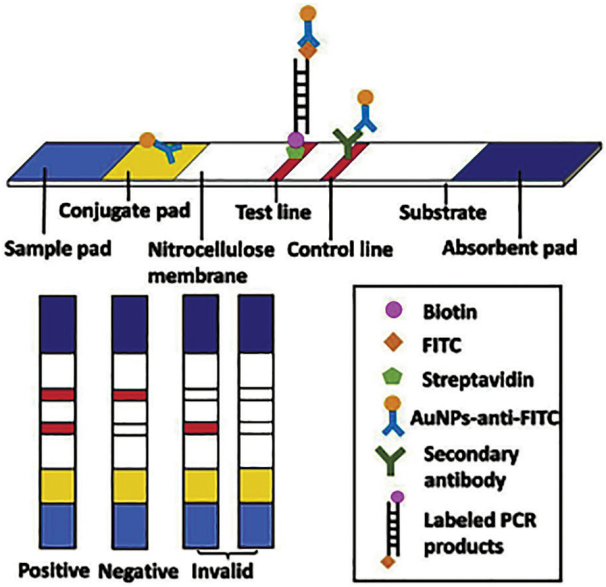

Figure 1. The example of a device for portable PCR test system [34]

Yin et al. developed a PCR test system in the form of a chromatographic strip for the quick identification of pork [35]. In the described test system, amplification and hybridization of amplicons with the probe were made outside the test strip. Visualization was achieved by test strips through hybridization of PCR resulted product. This test system detected an admixture of 0.01% pork in the tested product within 3 minutes (apparently, the authors mean the time without performing PCR).

To identify chicken meat in minced meat products, a method for the quick detection of counterfeit was developed [36]. The method was based on PCR combined with the use of a microfluidic chip. It featured a sensitivity of

0.1 pg for chicken DNA, and 0.1% for raw and autoclaved chicken meat in binary meat mix samples. The entire process of obtaining the result took 25 minutes — from sample preparation till getting the results [1,36]. Yin et al. developed a simple and quick test for identification of raw and cooked lamb using an immunochromatographic test strip [37]. After PCR the test mixture was applied to the test strip the test results were available in 5 minutes. This test system had a sensitivity of 0.01 pg for sheep DNA and 0.01% for the detection of adulterated meat. Qin et al. developed a quick and sensitive visual detection method for meat species identification [38]. Their method combined PCR and immunochromatographic analysis to detect duck meat admixtures in samples of beef. The method involved joint PCR amplification from the samples of beef and duck. This method made it possible to detect an admixture of foreign meat in amount of 0.05%. Magiati et al. developed a visual identification method based on immunochromatographic analysis for identification of horse meat and pork, as well as their binary mixtures with beef and lamb [39]. The authors used biotinylated primers for DNA amplification of different animal species' meat. Biotinylated amplicons were subjected to heat treatment to obtain single-stranded DNA. This single stranded DNA was hybridized with a complementary oligonucleotide probe that features a poly-A sequence at one end. The hybridized product was introduced into the conjugation zone of the immunochromatographic test strip. Poly-T sequences, immobilized on the T- and C-zones of DNA and bovine serum albumin, were used as reagents for biological determination of the presence of the sought-for hybridized structure. As a result of this procedure on the poly-A test system, the nucleic acid sections formed stable structures with poly-T sequences, thus making a visible color line. For this method, the analysis took from 25 to 30 minutes: the method had a sensitivity of 0.01% for horse DNA and 0.02% for pork DNA in binary meat mixtures.

LAMP (Loop-Mediated Isothermal Amplification) is a nucleic acid amplification method that does not require a thermal cycler. In this case DNA amplification runs at a constant temperature [1]. This method requires four or six primers to complete. Li et al. developed a test system that combined the LAMP method with immunochromatographic analysis [40]. At the first stage, isothermal amplification was run, and then the results were visualized on the test system in the form of a strip. The method made it possible to detect an admixture of 0.1% beef in a lump of minced meat, while the analysis took 50 minutes. Shi et al. developed a LAMP-based test system for the detection of duck DNA [41]. The method involved DNA amplification at a constant temperature of 65 °C for 30 min. The authors were able to achieve a sensitivity of 3 pg for duck DNA. The authors note that the sensitivity was higher than that of PCR. The authors also combined two varieties of the LAMP method (using two dyes as fluorescent marks).

Non-genetic devices for meat identification

Devices based on non-genetic methods of meat identification, as a rule, have simpler preparation of sample, and short time of analysis, but less specificity and lower sensitivity. As a rule, such test systems use the direct introduction of meat extract into a special zone on the test strip for immunochromatographic analysis. Most of these test systems use specific antibodies for meat proteins. Yayla et al. proposed a test system for pork detection in foods [42]. The authors report that their test system does not show cross-reactivity with beef, lamb, horse meat, mice and rabbit meat. When creating this test system, the authors conjugated antibodies with colloidal gold. Kuswandi et al. developed a quick test for detection of pork in ready-cooked meat food [43]. To do this, the developers obtained and conjugated gold nanoparticles with polyclonal immunoglobulin G. This test system detected pork admixture in amount of 0.1%. Masri et al. developed a test system for quick identification of horse meat [44]. In their test system, the authors used antibodies specific to horse serum albumin (HSA) and horse thermostable meat protein (H-TSMP). The method was able to detect 0.01% raw and 1.0% cooked horse meat in xenogeneic meat sources within 35 minutes. The method has demonstrated specificity in regards to serum albumin and meat derived from chicken, turkey, pig, cow, lamb and goat.

Meat identification methods that do not use chromatographic methods of analysis

Such test systems can demonstrate color detection of results in microtrays. Or these test systems can be, for example, run with the help of magnetic particles. So Seddaoui and Amine developed this test system [45]. It allowed detection of 0.01% pork admixture and was able to specifically detect pork among other types of meat (lamb, turkey, chicken and beef). Wu et al. developed a colorimetric system for detection of pork in binary meat mixtures [46]. A feature of this test system was that PCR was implemented in a glass capillary, and the results could be observed 20 minutes after the start of the test after hybridization. While the analysis SYTO 9 dye was used to visualize the PCR results. To detect pork in mixtures of beef and chicken meat, Skouridou et al. developed a test system using combined PCR and enzyme-immunoassay (PCR-ELONA test) [47]. As a result of the test, after hybridization of the PCR outcomes, the researchers observed color changes in the cells of the immunological tray, which made it possible to judge on the presence of pork admixtures in the analyzed samples. The sensitivity of the method ranged from 71 to 188 pg of genomic DNA. Lee et al. described a quick identification method using a portable colorimeter [48]. This method was based on the LAMP method and was able to detect 1 pg of pork DNA or 0.1% pork admixture in ground beef within 30 minutes. Wang et al. developed a LAMP-based method to detect trace amounts of horsemeat in foods [49]. This method could detect the presence

of 0.1% foreign meat and showed no cross-reactivity with 14 other animal species. Yan et al. developed a test to identify duck meat admixture [50]. The authors used the SEA method as the base of the test system. This quick method could find 10 pg/μl duck DNA or 0.1% duck meat in binary mixtures. The results of the test were available after 1 hour, and in this test no DNA extraction step was required (but still there was a cell lysis step during the analysis). The authors note that the reading of the results in their method can be implemented without special tools or instruments, and can be done visually (Figure 2).

Wang et al. also developed a test system based on SEA [51]. Their test system completed the analysis in 50 minutes and allowed to detect 1% beef admixture in the beef-duck mixture. A similar system was developed by Liu et al. for pork identification [20]. The idea of the method was to determine the presence of a mitochondrial DNA sequence specific for certain species. The authors note that their method was quick and could detect as little as 30 pg/μl of pork DNA. This method together with a colorimetric (fluorescence) detection method was able to find an admixture of 1% pork in a binary mixture. The proposed method included a protocol of quick DNA isolation and took 1 hour [20]. Montowska et al. analyzed the possibility of using a proteome to identify different types of meat [14]. As a result suitable protein markers were found to identify a particular type of meat before and after heat treatment (Figure 3).

In Figure 3 the identified proteins are marked with colored labels. The proteome gives a specific pattern made of separated proteins. That allows distinguishing one type of meat from another. Figure 3 shows that marker proteins are present on the proteome after heat treatment

Prospects for further development

The designing of new antibodies by genetic engineering methods can lead to higher specificity of portable test systems assigned for meat identification. On the other

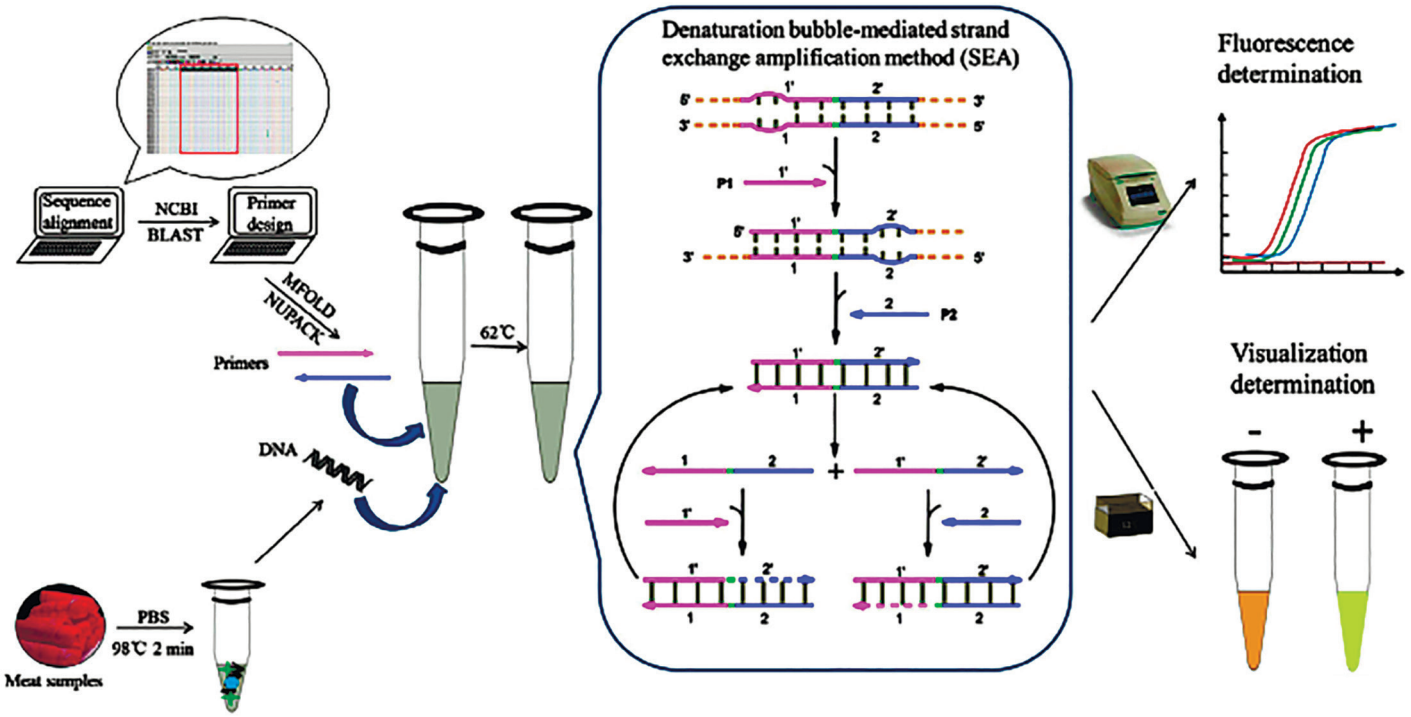

Figure 2. The example of a scheme for identifying the presence of foreign admixtures in a semi-finished meat product using SEA [50]

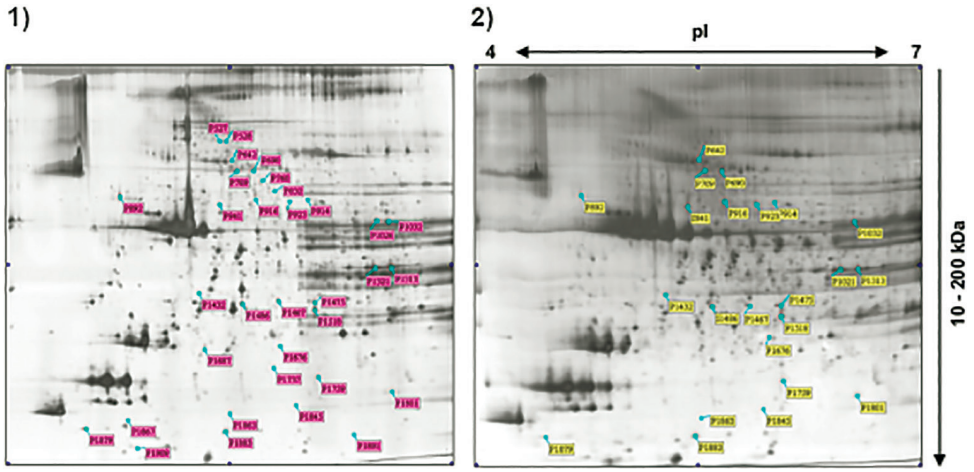

Figure 3. The example of a proteome obtained with the help of gel electrophoresis: 1 — raw pork, 2 — heat-treated pork [14]

hand, the emergence of new types of sorbents for thin layer chromatography will also contribute to a better separation of the components being under study, which sorbents will improve the performance of the developed test systems. One of the promising ways to develop test systems based on the study of nucleotide sequences is the use of isothermal amplification. The development of microfluidic technologies can lead to emergence of portable test systems for meat identification that combine several methods, for example: purification of meat extract and subsequent separation of the meat extract components on sorbent bedding.

Conclusion

Meat identification often requires bulky laboratory equipment, highly qualified lab-personnel, and a relatively time-consuming process of analysis. Meanwhile the possibility of quick identification without sophisticated molecular biological studies is often important. Therefore, the development of quick testing systems is naturally determined. Typically, quick test systems based on immunochromatographic methods have a shorter analysis time compared to test systems based on DNA sequences, and are more suitable for creating portable quick systems. However, the combination of methods for studying DNA, RNA and methods of enzyme-immunoassay and immunofluorescence analysis with thin layer chromatography makes it possible to create new species identification methods that feature both high speed of analysis and high sensitivity, moreover these new methods are quite compact to do. The development of molecular biology methods contributed to emergence of new methods for identification of meat species, for example, various types of isothermal amplification appeared. It is known that isothermal amplification, for example, LAMP, has a shorter analysis time in comparison with PCR and requires simpler equipment for its implementation; however at the same time it possesses a sensitivity and specificity comparable to PCR.

Thus, we observe a trend towards dimensions reduction of the test systems used for identification of meat species, meanwhile maintaining high sensitivity and high specificity. Probably in the future, isothermal amplification will be introduced even more widely, because it is a sensitive and specific method with a high speed of analysis. At the same time, it is likely that if successful detection systems are created for isothermal amplification, for example, colorimetric ones that do not require additional equipment, then they will probably push out the methods that use thin layer chromatography. In this case the parameters of such test systems are likely to be comparable with PCR-based identification methods. Meanwhile methods based on sequencing of genomic sequences are likely to be used in laboratories, but as the most accurate and non-portable test method that requires a lot of time.

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