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PROFESSIONAL REVIEW

Polymerase chain reaction in meat species identification

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Abstract

Food is considered authentic or genuine if the product or its contents conform to the original condition and label claims. Deviations from assertions and information on the declaration are considered non-compliances with food regulations. Due to the increased awareness of the problem and negative impact of food fraud, meat consumption is significantly influenced by consumer perceptions of food quality and safety. Therefore, accurate labelling is one of the most important factors influencing consumer preferences in the selection and purchase of meat and meat products. For this reason, analytical methods to verify the authenticity of meat and meat products are important to ensure product quality, food safety and consumer protection. The substitution of meat variety is not the only criterion for determining the authenticity of meat and meat products, but also the origin of the meat, the treatment of the meat and the addition of non-meat ingredients. Polymerase chain reaction (PCR) and its derived technologies have been shown to be the most suitable methods for species identification in raw and technologically processed meat. PCR methods are mainly based on the identification of the target region of mitochondrial DNA, which allows the detection of species in a wide range of meat products, including all domestic animals as well as game meat intended for human consumption. However, they also have some drawbacks. For example, random amplified polymorphic DNA (PCR-RAPD) is not suitable for species identification in meat mixtures as well as in thermally processed meat. On the other hand, some methods are expensive, time consuming and there are difficulties in interpreting the results. In this article, the main PCR-based methods for meat species identification are presented and described.

Keywords: meat species identification; PCR; meat fraud; food authenticity

Introduction

Preferences in the consumption of certain types of meat and meat products depend on culture, religious beliefs, and traditions (Bernabéu et al., 2018; Lorenzo et al., 2019; Maison et al., 2019; Meixner et al., 2018; Purslow et al., 2017). Along with it, the globalisation of the food industry and greater consumer awareness have increased awareness of the problem and negative impact

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of food adulteration. (Lorenzo et al., 2019; Xing et al., 2019). Therefore, the information on the declaration, especially the information on food safety, nutritional value, and quality attributes, has a great impact on consumer satisfaction. Therefore, correct labelling is one of the most important factors influencing consumer choice.

Food fraud in the form that the composition deviates from the standard composition, in relation to the food name of the declared values, deprives the consumer in financial, health and ethical terms (Koprivnjak, 2014). Adulteration associated with meat and meat products includes the fraudulent substitution or addition of low-quality animal proteins or the addition of cheap protein concentrates of plant origin (e.g., soy, pea, wheat; Cavin et al., 2018; Keyvan et al., 2017). Another common feature is mislabelling and the use of lower amounts of meat than indicated (Keyvan et al., 2017). In addition, cheaper raw materials such as offal (liver, kidneys, etc.), collagen or hydrolysed proteins produced from leather industry residues or animal remains (feathers, horns) could be used to replace and/or supplement muscle tissue (Gatmaitan et al., 2021; Grundy et al., 2016; Wagner et al., 2020). Meat could also be adulterated by the addition of various additives (Visciano and Schirone, 2021). Therefore, meat identification is essential for food quality control, food safety assurance and consumer protection.

The development of methods for the synthesis and analysis of nucleic acids had a great influence on the development of methods for the identification of individual animal species, which in turn influenced the implementation and development of methods for product authentication (Gvozdanović et al., 2017). PCR is a rapid, simple and specific method that allows amplification of a specific part of the DNA molecule and allows easy characterization and comparison of genetic material between different individuals and organisms. One of the main advantages of DNA molecule-based methods is their high discriminatory power and sensitivity, which allows the detection of 0.1% or even lower amounts of a specific meat species in a food product (Chung, 2018; Kumar et al., 2015; Li et al., 2021). The unique features of the DNA molecule allow the identification of different and related animal species, not only in raw meat products, but also in products that have been subjected to heat treatment and/or treated with various chemical compounds, and even in rendered animal products (Kaltenbrunner et al., 2018; Kang and Tanaka, 2018; Kumar et al., 2015; Li et al., 2020; Ruiz-Valdepeñas Montiel et al., 2017; Spychaj et al., 2009; Xu et al., 2018). The most commonly used PCR-based methods for meat and meat product authentication are multiplex PCR, restriction fragment length polymorphism (PCR-RFLP), random amplified polymorphic DNA (PCR-RADP), species-specific PCR, single-strand conformation polymorphism (PCR-SSCP), and real-time PCR (q-PCR). Overall, the aim of this review was to describe the principle, advantages and disadvantages of the PCR methods most commonly used in meat species identification.

PCR methods

The PCR reaction allows amplification of a specific nucleotide sequence of the initial DNA sample under in vitro conditions. In meat species identification, the target genes and DNA fragments used as molecular markers are mainly derived from mitochondrial DNA, e.g. D-loop, cytochrome b sequences, cytochrome c oxidase subunit I, II and III genes, ATPase subunit 6 and 8, 12SrRNA and 16SrRN (Li et al., 2020). There are several ways to verify the authenticity of meat and meat products using PCR technique. The simplest strategy for assessing the presence of a species consists of a three-step cycle of DNA fragment synthesis (denaturation, primer addition, and elongation) followed by gel electrophoresis, sequencing, or hybridization (Spychaj et al., 2009). Additional conformational methods and/or analyses of fragments can be performed using the other most commonly used PCR-based methods, e.g. multiplex PCR, PCR-RFLP, PCR-RAPD, species-specific PCR, PCR-SSCP and RT-PCR. Different protocols for the above mentioned methods have been developed for efficient amplification of variable regions within an ample range of organisms (Table 1), while their application in authenticity assessment of meat and meat products is reflected in Table 2.

Multiplex PCR

Multiplex PCR consists of a mixture of primer pairs that allows the simultaneous amplification of multiple DNA targets in a single reaction tube, with the products identified on agarose gels (Shen, 2019; Wu et al., 2018). It can be designed as a single PCR reaction using multiple primer sets

Table 1 PCR-based techniques developed for animal species identification

Reference	Method applied	Identified species
Liu et al., 2019 Wu et al., 2018	multiplex PCR	dog, chicken, cattle, pig, horse, donkey, fox, rabbit fox, dog, mink, rabbit
Al. et al., 2020 Gargouri et al., 2021 Vaithiyanathan et al., 2021	PCR-RFLP	cattle, water buffalo, horse, donkey dromedary, rabbit, goat, turkey, rat, donkey, pork camel
Jin et al., 2006 Koh et al., 1998 Lin et al., 2019	PCR-RAPD	freshwater salmonids wild boar, rabbit, kangaroo, pig, horse, bison, cow Holstein, Angus, Taiwan Yellow cattle
Amaral et al., 2014 Karabasanavar et al., 2017	species-specific PCR	hare, rabbit, red deer, pork, cow beef
Badi et al., 2021 Sivaraman et al., 2019	PCR-SSCP	sheep, goats, Japanese quails, African ostriches snapper species
Aina et al., 2019 Chen et al., 2020 Orbayinah et al., 2019	*RT-PCR*	wild boar beef pork

to amplify specific regions, or as a reaction using multiple regions and multiple primer sets in the same reaction tube. In addition, multiplex PCR can be a final or preliminary analysis that precedes hybridization or sequencing (Lees, 2003; Shen, 2019). Simultaneous amplification preserves time and resources for test preparation as well as the risk of cross-contamination due to minimal sample handling. Although optimising conditions can sometimes be time consuming, this method allows for primer and polymerase conservation and provides great flexibility in experimental design as well as overcoming limited primer kinetics and fragment competition (Shen, 2019).

Restriction fragment length polymorphism (PCR-RFLP)

PCR-RFLP is based on the DNA fragment cleavage reaction by restriction endonucleases to detect polymorphisms, which involve changes in the DNA sequence in a single nucleotide (Hashim and Al-Shuhaib, 2019; Rasmussen, 2012). PCR-RFLP represents a rapid and sensitive method that has high reproducibility and allows discrimination between closely related species, as well as discrimination between different species (Chappalwar et al., 2020; Pascoal et al., 2004). Although PCR-RFLP is one of the most important molecular methods for meat species identification, the method is complex, requires a properly equipped laboratory and expensive enzymes, while the enzymatic process is prone to incomplete digestion and leads to unreliable results (Alikord et al., 2018; Kumar et al., 2015; Li et al., 2020). Moreover, the technique is not suitable for the simultaneous analysis of a large number of polymorphisms due to the different requirements of primers and restriction enzymes (Rasmussen, 2012).

Random amplified polymorphic DNA (PCR-RAPD)

RAPDs are randomly amplified DNA segments produced by a PCR reaction using short primers. The analytical procedure involves isolation of DNA and its amplification by PCR reaction followed by electrophoretic separation and visualization of polymorphisms (Kumari and Thakur, 2014). The main advantage of PCR-RFLP is that the method does not require prior knowledge of the species to be identified. The method is sensitive, inexpensive and does not require expensive and highly specialized equipment. On the other hand, due to the high sensitivity to reaction conditions, there is a lack of reproducibility that sometimes cannot be compensated by standardization. Moreover, since short, randomly distributed primers can amplify DNA fragments from a large number of organisms, there is a risk of DNA contamination. On the other hand, gel electrophoresis allows quantitative (by size) but not qualitative (by nucleotide sequence) separation of fragments. The application of the PCR-RAPD method is limited in intensively processed products and meat mixtures due to the specific reaction conditions and DNA fragmentation (Chappalwar et al., 2020; Kumari and Thakur, 2014).

Table 1 PCR-based techniques in meat authenticity assessment

Reference	Region	Type and number of analysed samples	Declared meat species	Adulterants	% of adulterated products	Method applied
Keyvan et al., 2017	Turkey	sucuk (11), sausage (5), salami (3)	beef	poultry (chicken/ turkey), horse	sucuk (13.5 %), sausage (6.06 %), salami (21.8 %)	conventional PCR
Shehu et al., 2018	Albania	raw meatballs (20), sausages (40)	beef, chicken, pork	chicken, pork	bovine raw meatballs (100 %), chicken sausages (53.8 %), pork sausages (65 %)	conventional PCR
Liu et al., 2019	China	sausage (15), ball (8), meat muffins (5), beef products (21), pork products (15), chicken products (10), other meat products (29)	pork, chicken, beef, duck, dog, horse, donkey, rabbit, mutton	chicken, pork, rat, beef, dog, horse, donkey, rabbit, duck	sausage (53 %), ball (50 %), meat muffins (60 %), beef products (38 %), pork products (26.7 %), other meat products (10.3 %)	multiplex PCR
Galal-Khallaf, 2021	Egypt	smoked salami (6), dried salami (10),cooked salami (20), roast beef (20), beef bacon (10), luncheon meats (14)	beef	poultry	salami samples (61.6 %), luncheon meats (100 %)	multiplex PCR
Taha et al., 2021	Iraq	escallop (4), nugget (4), steak (4),sausage (4)	poultry	undeclared species	sausage (100 %)	PCR-RFLP
Farshidi et al., 2019	Iran	hamburger (15), salami (6), minced meat (10)	beef	chicken	hamburgers (61.6 %), salami (100 %), minced meat (80 %)	PCR-RFLP
Yacoub and Sadek, 2017	Saudi Arabia	luncheon meat, burgers	chicken	pork	-	species-specific PCR
El-Razik et al., 2019	Egypt	burger (24), minced meat (16), kofta (24), sausage (16), raw meat (7), luncheon (9)	beef	horse, donkey	minced meat (12.5 %), kofta (4.16 %), sausage (15.6 %)	species-specific PCR
Amaral et al., 2014	Portugal	Alheira (18)	game meat (rabbit, hare, red deer)	cow	Alheira (50 %)	species-specific PCR
Csikós et al., 2015	Hungary	meat products (12), cheese products (6)	chicken, turkey, pork, goose, duck, goat, sheep cattle	chicken, turkey, duck, goat, sheep, cattle	meat products (41.6 %), cheese products (50 %)	PCR-SSCP
Dalsecco et al., 2018	Brazil	hamburger (4), sausage (5), salami (1), smoked chicken breast (1), Canadian bacon (1), beef meatballs (1), nuggets (1)	lamb, beef, pork, chicken	beef, chicken, pork	total samples (42.8 %)	RT-PCR

Species-specific PCR

Species-specific PCR is based on the use of conserved or specifically designed primers that allow direct identification of defined DNA segments with sufficient species-specific variation. It is a rapid, accurate and highly sensitive method without the need for further sequencing or cleavage of PCR products. In order to design primers, it is necessary to know the nucleotide sequence of the gene that serves as the base. In other words, it is necessary to suspect the presence of a particular meat species in the sample before performing an analysis. This method can be applied to the analysis of a large number of samples, and of particular importance is its application to heat-treated foods (Alikord, 2018; Chappalwar, 2020; Karabasanavar et al.; Spychaj, 2009).

Single-strand conformation polymorphism (PCR-SSCP)

PCR-SSCP is a technique in which segments of DNA are amplified using specific primers. Then, the single-stranded DNA molecules are put into gel electrophoresis to determine the differences in nucleotide sequences. The main concept of PCR-SSCP is based on the fact that the electrophoretic mobility of nucleic acids in a non-denaturing gel depends on their shape and size (Badi et al., 2021; Hashim and Al-Shuhaib, 2019). Thus, the ability to detect mutations depends on how the mutation affects bending and how bending alone affects electrophoretic mobility (Hayashi, 1992). Although the method PCR-SSCP can be applied to many genes and many organisms, optimization steps are required because the main difficulty in genotyping PCR products is related to the exact conformation of a DNA fragment, which cannot be determined under the influence of variable parameters (e.g., amplicon sizes, porosity of gels, etc.). Moreover, before loading PCR amplicons into the polyacrylamide gels, various pre-electrophoresis steps must be performed (Badi et al., 2021).

Real time PCR (RT-PCR)

The RT-PCR method is performed by monitoring the fluorescence signal, which allows inference of the initial amount of target genes without additional steps (Xu et al., 2018). The results of RT-PCR method can be qualitative, indicating the presence or absence of DNA sequences, and quantitative, indicating the initial concentration of the DNA molecule. SYBR Green and TaqMan technology are widely used for quantitative detection of DNA (Kumar et al., 2015; Li et al., 2020). In addition, amplification and analysis of results in "real time" reduces the need for manipulation after cycles have been performed, thus reducing the possibility of sample contamination (Shen, 2019). In the RT-PCR design, the purity, quality, and concentration of the DNA template affect the quantitative results. The single-stranded DNA, RNA, and DNA polymerase could cause false positive or false negative results (Kang and Tanaka, 2018; Li et al., 2020; Xu et al., 2018). However, the current application of RT - PCR in research is limited by the relatively high cost of reagents and equipment, and the complexity of design and optimization (Liu et al., 2019; Safdar and Junejo, 2015).

Conclusion

The authenticity and safety of meat and meat products is a major concern for consumers and especially the meat industry. Identification of meat species is important not only for consumer confidence in food products, but also to ensure their protection in financial, health and ethical terms. Nowadays, PCR-based methods for species identification have replaced other conventional techniques. They represent rapid, reliable, specific and sensitive methods for authenticity testing. Thanks to the properties of the DNA molecule, PCR-based methods allow species identification in raw and processed foods. They also allow differentiation between different and within the same species. However, their applicability depends on the nature of the sample and the requirements of the tests to be performed. PCR-RAPD is not applicable to intensively processed products and meat mixtures, while species-specific PCR requires knowledge of the nucleotide sequence of the target species. In addition, methods such as PCR-RFLP and RT-PCR require sophisticated equipment. Nevertheless, the use and further development of PCR-based methods for meat authentication will continue to increase.

Literatura

[1] **Aina, G.Q., Y. Erwanto, M. Hossain, M.R. Johan, M.E. Ali, A. Rohman (2019):** The employment of q-PCR using specific primer targeting on mitochondrial cytochrome-b gene for identification of wild boar meat in meatball samples. J Adv Vet Anim Res 6(3): 300 – 307.

[2] **Al, S., H. Hizlisoy, N. Ertaş Onmaz, F. Karadal, C. Güngör, Y. Yildirim, Z. Gönülalan (2020):** The determination of meat species by PCR-RFLP method using mitochondrial ND4 gene in pastırma, a traditional dry cured meat product. Turkish J Vet Anim Sci 44: 35 – 41.

[3] **Alikord, M., H. Momtaz, J. Keramat, M. Kadivar, A.H. Rad (2018):** Species identification and animal authentication in meat products: a review. Food Measure 12: 145 – 155

[4] **Amaral, J.S., C.G. Santos, V.S. Melo, M.B.P.P. Oliveira, I. Mafra (2014):** Authentication of a traditional game meat sausage (Alheira) by species-specific PCR assays to detect hare, rabbit, red deer, pork and cow meats. Food Res Int 60:140–145.

[5] **Badi, M.A., M.B. Al-Shuhaib, T.R. Aljubouri, T.M. Al-Thuwaini, H.H. Dawud, T.H. Hussein, A.T. Al-Nafii, D. Abdulmalek, M.K. Altamemi, I.A. Fadhil, A.H. Albakri, H.O. Hashim, H.O., A.K. Mohammed (2021):** Rapid and optimized protocol for efficient PCR-SSCP genotyping for wide ranges of species. Biologia 76:2413–2420.

[6] **Bernabéu, R., A. Rabadán, N.E. El Orche, M. Díaz (2018):** Influence of quality labels on the formation of preferences of lamb meat consumers. A Spanish case study. Meat sci 135: 129 – 133.

[7] **Cavin, C., G. Cottenet, K.M. Cooper, P. Zbinden (2018):** Meat vulnerabilities to economic food adulteration require new analytical solutions. Chimia 72 (10): 697 – 703.

[8] **Chappalwar, A.M., V. Pathak, M. Goswami, B. Sharma, P. Singh, R. Mishra (2020):** Recent novel techniques applied for detection of meat adulteration and fraudulent practices. ndian J Vet Public Hlth 7(1): 1 – 6

[9] **Chen, X., L. Lu, X. Xiong, X. Xiong, Y. Liu (2020):** Development of a real-time PCR assay for the identification and quantification of bovine ingredient in processed meat products. Sci Rep 10: 2052.

[10] **Chung, H.H. (2018):** Real-time polymerase chain reaction (RT-PCR) for the authentication of raw meats. Int Food Res J 25(2): 632 – 638.

[11] **Csikós, Á., Á Tisza, Á Simon, G. Gulyás, A. Jávor, L. Czeglédi (2015):** Species identification in meat and cheese products by PCR-single strand conformation polymorphism (PCR-SSCP) and DNA sequencing. AWETH 11(2): 78 – 83.

[12] **Dalsecco, L.S., R.M. Palhares, P. C. Oliveira, L.V. Teixeira, M. G. Drummond, D.A. A. de Oliveira (2018):** A fast and reliable real-time PCR method for detection of ten animal species in meat products. Journal of Food Science, 83(2), 258 – 265.

[13] **El-Razik, K.A.E.H., A.S.M. Abuelnaga, A.M. Younes, N.S. Atta, A.A. Arafa, M.M. Kandil (2019):** Species – specific PCR test for the quick recognition of equine tissue in raw and processed beef meat mixtures. Food Sci Technol 39:166–172.

[14] **Farshidi, M., B. Ebrahimi, R. Mohammadi, M.R. Sehatkhah, J. Moludi, G. Moghaddam (2019):** Identification of mislabeling some meat products sold on the Iran market using PCR-RFLP. Curr Nutr Food Sci 15: 1 – 5

[15] **Galal-Khallaf, A. (2020):** Multiplex PCR and 12S rRNA gene sequencing for detection of meat adulteration: A case study in the Egyptian markets. Gene 764: 145062

[16] **Gargouri, H., N. Moalla, H.H. Kacem (2021):** PCR–RFLP and species-specific PCR efficiency for the identification of adulteries in meat and meat products. Eur Food Res Technol 247: 2183–2192

[17] **Gatmaitan, A.N., J.Q. Lin, J. Zhang, L. S. Eberlin (2021):** Rapid analysis and authentication of meat products using the MasSpec Pen technology. J Agric Food Chem 69(11): 3527 – 3536.

[18] **Grundy, H.H., P. Reece, M. Buckley, C.M. Solazzo, A.A. Dowle, D. Ashford, A.J. Charlton, M.K. Wadsley, M.J. Collins (2016):** A mass spectrometry method for the determination of the species of origin of gelatine in foods and pharmaceutical products. Food Chem 190: 276 – 284.

[19] **Gvozdanović, K., P. Margeta, V. Margeta (2017):** Primjena analitičkih metoda u postupcima autentifikacije poljoprivrednih i prehrambenih proizvoda. Stočarstvo 1: 29 – 38.

[20] **Hashim, H.O., M.B.S. Al-Shuhaib (2019):** Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: a review. J Appl Biotechnol Rep 6(4): 137 – 144

[21] **Hayashi K. (1992):** PCR-SSCP: A method for detection of mutations. GATA 9(3): 73 – 79.

[22] **Jin, L.G., J.Y. Cho, K.B. Seong, J.Y. Park, I.S. Kong, Y.K. Hong (2006):** 18S rRNA gene sequences and random amplified polymorphic DNA used in discriminating Manchurian trout from other freshwater salmonids. Fish Sci 72: 903–905.

[23] **Kaltenbrunner, M., R. Hochegger, M. Cichna-Markl (2018):** Tetraplex real-time PCR assay for the simultaneous identification and quantification of roe deer, red deer, fallow deer and sika deer for deer meat authentication. Food Chem 269: 486–494.

[24] **Kang, T. S., T. Tanaka (2018):** Comparison of quantitative methods based on SYBR Green real-time qPCR to estimate pork meat adulteration in processed beef products. Food Chem 269: 549–558.

[25] **Karabasanavar, N., P.S. Girish, D. Kumar, P. Singh (2017):** Detection of beef adulteration by mitochondrial D-loop based species-specific polymerase chain reaction. Int. J Food Prop 20(2): 2264–2271.

[26] **Keyvan, E., C.G. İplikçioğlu, B.C. Kul, N. Bilgen, U.T. Şireli (2017):** Identification of meat species in different types of meat products by PCR. Vet Fak Derg 64: 261 – 266.

[27] **Koh, M.C., C.H. Lim, S.B. Chua, S.T. Chew, S.T.W. Phang (1998):** Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci 48 (3-4): 275–285.

[28] **Koprivnjak, O. (2014):** Kvaliteta, sigurnost i konzerviranje hrane. Rijeka, Medicinski fakultet Rijeka.

[29] **Kumar, A., R.R. Kumar, B.D. Sharma, P. Gokulakrishnan, S.K. Mendiratta, D. Sharma (2015):** Identification of species origin of meat and meat products on the DNA Basis: A Review. Crit Rev Food Sci Nutr 55(10), 1340 – 1351

[30] **Kumari, N., S.K. Thakur (2014):** Randomly amplified polymorphic DNA-A brief review. Am J Anim Vet Sci 9 (1): 6 – 13

[31] **Lees, M. (2003):** Food authenticity and traceability. Abington, Cambridge. Woodhead Publishing, 2003.

[32] **Li, J., J. Li, R. Liu, Y. Wei, S. Wang (2021):** Identification of eleven meat species in foodstuff by a hexaplex real-time PCR with melting curve analysis. Food Control 121: 107599.
[33] **Li, Y.C., S.Y. Liu, F.B. Meng, D.Y. Liu, Y. Zhang, W. Wang, J.M. Zhang (2020):** Comparative review and the recent progress in detection technologies of meat product adulteration. Compr Rev Food Sci Food Saf 19, 2256 – 2296
[34] **Lin, C.-C., P.-C. Tang, H.-I Chiang (2019):** Development of RAPD-PCR assay for identifying Holstein, Angus, and Taiwan Yellow Cattle for meat adulteration detection. Food Sci Biotechnol 28(6):1769–1777.
[35] **Liu, W., J. Tao, M. Xue, J. Ji, Y. Zhang, L. Zhang, W. Sun (2019):** A multiplex PCR method mediated by universal primers for the identifcation of eight meat ingredients in food products. Eur Food Res Technol 245: 2385 – 2392
[36] **Lorenzo, J.M., P.E.S. Munekata, F.J. Barba, F. Toldrá (2019):** More than beef, pork and chicken – the production, processing, and quality traits of other sources of meat for human diet. Switzerland; Springer Nature.
[37] **Maison, D., M. Marchlewska, R.A. Zein, D. Syarifah, H. Purba (2019):** Religiously permissible consumption: The influence of the halal label on product perceptions depending on the centrality of religiosity. JIMA 10(3): 948 – 960.
[38] **Meixner, O., R. Friedl, B. Hartl (2018): Preferences for attributes of halal meat:** empirical evidence from the muslim community in Vienna, Austria. Int J Food Syst Dyn 9(3): 265 – 278.
[39] **Orbayinah, S., H. Widada, A. Hermawan, S. Sudjadi, A. Rohman (2019):** Application of real-time polymerase chain reaction using species specific primer targeting on mitochondrial cytochrome-b gene for analysis of pork in meatball products. J Adv Vet Anim Res 6(2): 260 – 265.
[40] **Pascoal, A., M. Prado, J. Castro, A. Cepeda, J. Barros-Velázquez (2004):** Survey of authenticity of meat species in food products subjected to different technological processes, by means of PCR-RFLP analysis. Eur Food Res Technol 218: 306 – 312
[41] **Purslow, P., R. Cepeda, E. Cáffaro, L. Garitta, M. Sosa, D. Frank, L. Duizer, T. Ngapo, H. Bruce, R. Nassu, M. Henchion (2017):** Differences in consumer perceptions of meat quality. 63rd International Congress of Meat Science and Technology. Cork, August 2017, Proceedings pp 766 – 767.
[42] **Rasmussen, H.B. (2012):** Restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) and gel electrophoresis - valuable tool for genotyping and genetic fingerprinting. In: Gel Electrophoresis/ Magdeldin Sameh (ed.). UK, IntechOpen, 2012.
[43] **Ruiz-Valdepeñas Montiel, V., M.L. Gutiérrez, R.M. Torrente-Rodríguez, E. Povedano, E. Vargas, A.J. Reviejo, R. Linacero, F.J. Gallego, S. Campuzano, J.M. Pingarron (2017):** Disposable amperometric polymerase chain reactionfree biosensor for direct detection of adulteration with horsemeat in raw lysates targeting mitochondrial DNA. Anal Chem 89: 9474–9482.
[44] **Safdar, M., Y. Junejo (2015):** Development and validation of fast duplex real-time PCR assays based on SYBER Green florescence for detection of bovine and poultry origins in feedstuffs. Food Chem 173(C):660–664.
[45] **Shen, C.H. (2019):** Amplification of nucleic acids. In: Diagnostic molecular biology/ Shen, Chang-Hui (ed.). Massachusetts, US; Academic Press, 2019
[46] **Sivaraman, B., G. Jeyasekaran, R.J. Shakila, S. Aanand, D. Sukumar (2019):** Authentication of nine snapper species by single-strand conformation polymorphism (SSCP) and forensically informative nucleotide sequencing (FINS) methods. Food Control 99:124–130.
[47] **Shehu, F., A. Di Pinto, P. Marchetti, M. Tantillo, B. Bijo, E. Nexhipi (2018):** Detection of species substitution and mislabeling in Albanian meat products by identification of DNA. Albanian j agric sci Proceedings of ICOALS, 182 – 187.
[48] **Spychaj, A., P.E. Mozdziak, E. Pospiech (2009):** PCR methods in meat species identification as a tool for the verification of regional and traditional meat products. Acta Sci Pol, Technol Aliment 8(2), 5 – 20
[49] **Taha, K.M., D.M. Khdr, K.Y. Kareem (2021):** Identification and differentiation of poultry meat and products using PCR-RFLP technique. MJA 49(1): 34 – 42
[50] **Vaithiyanathan, S., M.R. Vishnuraj, G. Narender Reddy, C. Srinivas (2021):** Authentication of camel meat using species-specific PCR and PCR-RFLP. J Food Sci Technol 58: 3882 – 3889
[51] **Visciano, P., M. Schirone (2021):** Food frauds: global incidents and misleading situations. Trends Food Sci Technol 114: 424 – 442.
[52] **Wagner, L., M. Peukert, B. Kranz, N. Gerhardt, S. Andrée, U. Busch, D.A. Brüggemann (2020):** Comparison of targeted (HPLC) and nontargeted (GC-MS and NMR) approaches for the detection of undeclared addition of protein hydrolysates in turkey breast muscle" Foods 9 (8): 1084.
[53] **Wu, Q., S. Xiang, W. Wang, J. Zhao, J. Xia, Y. Zhen, B. Liu (2018):** Species identification of fox-, mink-, dog- and rabbit-derived ingredients by multiplex PCR and real-time PCR assay. Appl Biochem Biotechnol 185, 1 – 12
[54] **Xing, R.-R., N. Wang, R.-R. Hu, J.-K. Zhang, J.-X. Han, Y. Chen (2019):** Application of next generation sequencing for species identification in meat and poultry products: A DNA metabarcoding approach. Food Control 101, 173 – 179.
[55] **Xu, R., S. Wei, G. Zhou, J. Ren, Z. Liu, S. Tang, X. Wu (2018):** Multiplex TaqMan locked nucleic acid real-time PCR for the differential identification of various meat and meat products. Meat Sci 137:41–46.
[56] **Yacoub, H.A., M.A. Sadek (2017):** Identification of fraud (with pig stuffs) in chicken-processed meat through information of mitochondrial cytochrome b. DNA Mapp Seq Anal 28(6): 855 – 859.

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Lančana reakcija polimerazom u identifikaciji vrsta mesa

Sažetak

Hrana se smatra autentičnom kada proizvod ili njegov sadržaj odgovaraju izvornom stanju i podacima na deklaraciji. Odstupanja od tvrdnji i navoda na deklaraciji smatraju se nesukladnostima s propisima o hrani. Zbog pojačane svijesti o problemu i negativnim učincima patvorenja hrane, na konzumaciju mesa značajno utječe percepcija potrošača o kvaliteti i sigurnosti hrane, pri čemu je ispravno deklariranje jedan od najvažnijih čimbenika koji utječe na preferencije u vidu odabira i kupovine mesa i mesnih proizvoda. Zbog navedenog razloga, analitičke metode koje se upotrebljavaju za provjeru autentičnosti važne su radi osiguranja kvalitete proizvoda, sigurnosti hrane, ali i zaštite potrošača. Zamjena vrsta mesa nije jedini kriterij kojim se utvrđuje njegova autentičnost, te obuhvaća i podrijetlo mesa, način njegove prerade, kao i dodatak ostalih sastojaka. Lančana reakcija polimerazom (PCR) i njezine modifikacije prepoznate su kao najprikladnije metode za identifikaciju vrsta u sirovom i tehnološki prerađenom mesu. PCR metode uglavnom se temelje na identifikaciji ciljane regije mitohondrijske DNK, što omogućuje detekciju vrsta u širokom spektru mesnih proizvoda, uključujući sve domaće životinje, kao i meso divljači koje se upotrebljava u prehrani ljudi. Unatoč navedenome, PCR metode posjeduju određene nedostatke, te tako primjerice, PCR-RAPD nije prikladan za identifikaciju vrsta mesa u mješavinama, kao i termički prerađenog mesa, dok su neke metode skupe, dugotrajne i posjeduju poteškoće vezane uz interpretaciju rezultata. U ovom radu predstavljene su i opisane najvažnije metode koje se temelje na PCR tehnici, a upotrebljavaju se za identifikaciju vrsta mesa.

Key words: identifikacija vrsta mesa; PCR; patvorenje mesa; autentičnost hrane

Polymerase-Kettenreaktion zur Identifizierung von Fleischarten

Zusammenfassung

Lebensmittel gelten als authentisch, wenn das Produkt oder sein Inhalt mit dem Originalzustand und den Angaben auf der Deklaration übereinstimmt. Abweichungen von den Behauptungen und Angaben auf der Deklaration werden als Verstöße gegen die Lebensmittelvorschriften angesehen. Aufgrund des gestiegenen Bewusstseins für das Problem und die negativen Auswirkungen von Lebensmittelbetrug wird der Fleischkonsum maßgeblich von der Wahrnehmung der Verbraucher in Bezug auf die Lebensmittelqualität und Sicherheit beeinflusst. Daher ist eine genaue Etikettierung einer der wichtigsten Faktoren, die die Verbraucherpräferenzen bei der Auswahl und dem Kauf von Fleisch und Fleischerzeugnissen beeinflussen. Aus diesem Grund sind Analysemethoden zur Überprüfung der Echtheit von Fleisch und Fleischerzeugnissen wichtig, um die Produktqualität, Lebensmittelsicherheit und den Verbraucherschutz zu gewährleisten. Die Substitution von Fleischsorten ist nicht das einzige Kriterium für die Bestimmung der Echtheit von Fleisch und Fleischerzeugnissen, sondern auch die Herkunft des Fleisches, die Behandlung des Fleisches und der Zusatz von sonstigen Zutaten. Die Polymerase-Kettenreaktion (PCR) und die davon abgeleiteten Technologien haben sich als die am besten geeigneten Methoden zur Identifizierung von Arten in rohem und technologisch verarbeitetem Fleisch erwiesen. Die PCR-Methoden basieren hauptsächlich auf der Identifizierung der Zielregion der mitochondrialen DNA, was den Nachweis von Arten in einer breiten Palette von Fleischerzeugnissen ermöglicht, einschließlich aller Haustiere und des für den menschlichen Verzehr bestimmten Wildfleischs. Sie haben jedoch auch einige Nachteile. So eignet sich beispielsweise die zufällig amplifizierte polymorphe DNA (PCR-RAPD) nicht für den Artennachweis in Fleischmischungen und in thermisch verarbeitetem Fleisch. Andererseits sind einige Methoden teuer und zeitaufwendig, und die Ergebnisse sind schwer zu interpretieren. In diesem Artikel werden die wichtigsten PCR-basierten Methoden zur Identifizierung von Fleischarten vorgestellt und beschrieben.

Schlüsselwörter: Fleischartenbestimmung; PCR; Fleischbetrug; Lebensmittelechtheit

La reacción en cadena de la polimerasa en la identificación del tipo de carne

Resumen

Los alimentos se consideran auténticos cuando el producto o su contenido corresponde al estado original y a los datos de la declaración. Las divergencias de las declaraciones se consideran incumplimientos de las regulaciones alimentarias. Debido a la mayor conciencia sobre el problema y los efectos negativos de la falsificación de los alimentos, el consumo de carne está significativamente influenciado por las percepciones de los consumidores sobre la calidad y seguridad de los alimentos, siendo la declaración correcta uno de los factores más importantes que influyen sobre las preferencia a la hora de elegir y comprar la carne y los productos cárnicos. Por esta razón, los métodos analíticos utilizados para verificar la autenticidad son importantes para garantizar la calidad del producto, la seguridad alimentaria, pero también la protección del consumidor. La sustitución de tipos de carne no es el único criterio para determinar su autenticidad e incluye el origen de la carne, el método de procesamiento, así como la adición de otros ingredientes. La reacción en cadena de la polimerasa (PCR) y sus modificaciones son identificadas como los métodos más apropiados para identificar los tipos de carne en la carne cruda y la carne procesada tecnológicamente. Los métodos de PCR están basados principalmente en la identificación de las secuencias cortas de la ADN mitocondrial, lo que permite la detección de especies en una amplia gama de productos cárnicos, incluidos todos los animales domésticos, así como la carne de caza para el consumo humano. A pesar de esto, los métodos de PCR tienen ciertas deficiencias, por lo que, por ejemplo, el PCR - RAPD no es adecuado para identificar tipos de carne en mezclas, así como carne procesada térmicamente, mientras que algunos métodos son costosos, consumen mucho tiempo y dan resultados difíciles para interpretar. Este artículo presenta y describe los métodos más importantes basados en la técnica de PCR, que se utilizan para identificar los tipos de carne.

Palabras claves: identificación del tipo de carne; PCR; falsificación de la carne; autenticidad de los alimentos

Reazione a catena della polimerasi nell'identificazione della varietà di carne

Riassunto

Il cibo è considerato autentico o genuino quando il prodotto o il suo contenuto corrispondono alla condizione originale e ai dati dell'etichetta. Ogni deviazione dalle asserzioni e dalle indicazioni contenute nell'etichetta è considerata difformità rispetto alla normativa sul cibo. A causa della maggior consapevolezza rispetto al problema e riguardo agli effetti negativi del cibo contraffatto, sulla consumazione della carne incide significativamente la percezione del consumatore circa la qualità e la sicurezza del cibo, laddove la corretta etichettatura è uno dei fattori più importanti che incidono tanto sulle preferenze riguardo alla scelta, quanto sull'acquisto della carne e dei prodotti a base di carne. Per quanto detto, i metodi analitici che si utilizzano per verificare l'autenticità sono importanti ai fini di garantire non solo la qualità del prodotto e la sicurezza alimentare, ma anche la tutela dei consumatori. La sostituzione della varietà di carne non è l'unico criterio per accertarne l'autenticità, e comprende anche l'origine della carne, le modalità della sua lavorazione e, infine, l'aggiunta di altri ingredienti. La reazione a catena della polimerasi (PCR) e le sue modificazioni sono riconosciute come il metodo più idoneo all'identificazione delle varietà nella carne cruda e in quella sottoposta a processo tecnologico di lavorazione. I metodi PCR si basano, in linea di massima, sull'identificazione di una regione del DNA mitocondriale, il che consente la individuazione della varietà in una vasta gamma di prodotti a base di carne, compresi tutti gli animali d'allevamento e la carne degli animali selvatici che viene utilizzata nell'alimentazione umana. Nonostante ciò, i metodi PCR presentano determinate lacune e così, ad esempio, il PCR-RAPD non è idoneo all'identificazione della varietà di carne nei mix di carni, come anche nella carne termicamente trattata, mentre altri metodi sono costosi, hanno una durata eccessiva e presentano difficoltà legate all'interpretazione dei risultati. In questo studio vengono presentati e descritti i metodi più importanti che si basano sulla tecnica PCR, utilizzati per l'identificazione delle varietà di carne.

Parole chiave: identificazione delle varietà di carne; PCR; contraffazione della carne; autenticità del cibo