12. History, present and future of food authentication at DNA level. Basic assumptions for usability of genetic data

Today's topic will be dedicated to the History, present and future of food authentication at DNA level, the lecture is part of module number 1 - Animal Genetics, which is part of the ISAGREED project. This presentation was supported by the Erasmus plus KA2 Collaborative Partnership Grant - Innovating the structure and content of the curricula in the management of animal genetic and food resources using digitalization.

Food authentication is the process of verifying the authenticity, origin and quality of food products. It is an important tool in the fight against counterfeiting and fraud in the food chain. Food authentication helps to protect consumers, producers and traders from health, economic and environmental risks. Food authentication can be carried out using a variety of methods such as analytical, molecular, sensory or digital techniques.

Every cell in the body contains genetic information about the whole organism. This information is stored in DNA, which is found in the nucleus of the cell. So, to find out a lot of information about an organism, we only need to look into the nucleus of the cell and analyze different fragments of DNA. By examining the DNA, we can find out information that can be used in the process of food authentication.

Sanger sequencing is a DNA sequencing method that was first used in 1977. This method is based on the use of modified dideoxy nucleotides and became the standard for molecular biology research for the next 30 years. In 1996, Applied Biosystems launched the first commercial automated sequencer. However, further development of automated sequencers could no longer achieve the stated goal of sequencing the entire human genome under the $1,000 threshold. The period of monopoly of classical Sanger sequencing is also called Generation I sequencing and lasted until about 2005.

Microsatellites are short repetitive DNA sequences found in the genome of every individual. The use of microsatellites in the process of food identification at the individual level is based on PCR analysis followed by fragment analysis. Fragment analysis uses different variable loci such as AHT4 or LEX3 where significant variation is observed in the length of the repetitive sequence which can be identified by the length itself or, in standardised systems, by the letter.

Capillary electrophoresis is a separation method that allows the separation of microsatellite fragments based on their size and charge. The fragments are placed in a capillary filled with a gel medium and then exposed to an electric field. The fragments move within the capillary based on their charge and size. Smaller fragments move faster than larger fragments and therefore the fragments are separated according to their size. In the case where a sequencer is used to identify fragments, the fragments are fluorescently labeled and as they pass through the optical sensor, a rise in fluorescence is identified and assigned to a specific time, which is also used to identify size of the fragment.

Microarray is a technology that allows simultaneous measurement of gene expression for thousands of genes in a single experiment. This technology is used to study genetic variants and their relationship to various diseases. For food authentication purposes, it has been alternative

to identify different food animals and simplify analysis procedures. The representative of the modified macroarray technology is CHIPRON.

Food authentication using CHIPRON's MEAT5 chip has four phases.

The first phase uses isolated DNA from the food as template for the PCR reaction in which the primers used for targeting are biotin-labeled. Biotin is used as a label in the PCR reaction to detect the amplification product. The biotinylated primer is bound to a single strand of DNA and after amplification, the streptavidin enzyme is bound to the product, which is coupled to a detection system. The chip used in the analysis has sections of DNA immobilised at specific locations complementary to each species. In the hybridisation process, the biotinylated products of the PCR reaction are combined with the complementary DNA strands immobilised on the chip. Subsequent staining procedures stain these junctions to reveal in the sample the DNA of the specific species targeted by the chip. In subsequent analysis, the position of the colour change can reveal the presence of several food animal species at once.

Genomics has changed the way food is authenticated by allowing both producers and official control institutions to identify the source of raw materials. Genomics is used to identify plant species and varieties as well as animal species and breeds. It is also used to identify micro-organisms in food. Thanks to genomics, a food producer can now accurately determine which species of plants or animals were used in the production of a food and whether that food is authentic or adulterated.

NGS (next generation sequencing) and metabarcoding are used in food authentication to identify the type and origin of food. NGS allows the analysis of large volumes of data in a single test and is able to detect the composition of food at the molecular level. Metabarcoding is a method of identifying the type of food based on DNA sequences. Miniaturization of instruments and the development of the latest semiconductor sequencing methods have made these methods significantly cheaper and simplier and can therefore also be used in routine food diagnostics.

The metabarcoding procedure usually consists of several steps: DNA extraction, PCR amplification, sequencing and data analysis.

- DNA extraction involves the isolation of DNA from food. In most cases, a CTAB-based extraction method or a commercial DNA isolation kit is used.

- PCR amplification involves the amplification of target DNA using PCR (polymerase chain reaction). Specific primer sets are used to amplify target DNA that target the most variable sections of the genome of plants and animals.