SPECIES AND CULTIVAR DIFFERENTIATION FOR FOOD AUTHENTICATION - DEVELOPMENT OF ASSAYS BASED ON PCR AND HIGH-RESOLUTION MELTING

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Economically motivated food adulteration remains a global issue. One of the common practices is the replacement of higher-value species or cultivars with cheaper ones.

DNA-based methods play an increasing role in species and cultivar differentiation. A common approach is the analysis of evolutionary conserved DNA regions, so-called DNA barcodes.[1] More precisely, both the 5´ end and the 3´ end of the DNA barcode should be conserved, allowing for amplifying the DNA barcode with one single primer pair in all species/cultivars of interest. However, in between these conserved parts serving as primer binding sites, the DNA sequence should be variable, enabling species/cultivar differentiation. In many cases, DNA barcodes selected for food authentication contain single nucleotide polymorphisms (SNPs) or microsatellites (simple sequence repeats, SSRs).

In case the DNA barcode is amplified with a PCR mix containing an intercalating dye such as EvaGreen, the amplicons can subsequently be subjected to high-resolution melting (HRM).[2] By slowly increasing the temperature, the amplicons are dissociated (melted) into the two single strands and fluorescence decreases. For data evaluation, the (normalized) fluorescence signal is plotted against temperature. The melting behaviour of the amplicons depends on various parameters, including their length and the ratio of guanine and cytosine to adenine and thymine.

The development and optimization of assays based on PCR and HRM will be discussed on several examples, including the differentiation between 1) edible insect species approved in the EU[3]; 2) bilberry and blueberry; and 3) wine varieties from North Macedonia.

REFERENCES

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