CANNABIS SATIVA SEEDS HOMOGENIZATION WITH MINILYS FOR REAL-TIME PCR

PREPARE YOUR CANNABIS SAMPLES FOR REAL-TIME PCR

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CONTEXT

Cannabis plants have a long history of being used for recreational, medicinal, and religious purposes. Over the past decades, to counteract its use as a recreative drug, most world authorities have banned all *Cannabis* varieties without making a distinction based on the content of tetrahydrocannabinol (THC), the active principle responsible for *Cannabis* psychotropic effect. However, recently, some countries have authorized the cultivation of hemp (*Cannabis sativa*) cultivars characterized by a low amount of THC. Since then, the benefits of hemp have attracted widespread attention in the food and wellness industry. Hemp leaves are rich in CBD (cannabidiol) which has well-documented anxiolytic, spasmolytic, as well as anticonvulsant effects. On the other hand, hemp seeds exhibit a pleasant taste and are a valuable source of nutrients such as amino acids, fatty acids, and fibers.

Consequently, an increasing number of food producers have started using hemp as an ingredient in their products. To ensure consumer safety, reliable methods to verify and quantify the presence of *Cannabis sativa* (hemp) in food will be needed. In this study, a real-time PCR method was developed to allow the precise detection of infinitesimal hemp traces in food samples. Here, DNA extracted from hemp seeds with the Minilys homogenizer was used to determine the optimal primer and probe concentrations for this PCR method.

PROTOCOL

Homogenization: Composite samples of 5 hemp seeds belonging to the *Monoica* variety were ground with the Minilys tissue homogenizer (Bertin Technologies, Montigny-les-Bretonneux, France) using the Precellys MK28 R 2mL lysing kit (ref: P000917-LYSK0-A, Bertin Technologies, Montigny-les-Bretonneux, France).

DNA extraction: The DNA was extracted with the *NucleoSpin Plant II Kit* (Macherey-Nagel, Germany). Based on the measured concentration, the extracts were diluted to an operating concentration of 5 ng/µL for subsequent PCR analysis.

Real-time PCR:

Primers and probes: A primer/probe set was designed to target the hemp-specific spacer DNA sequence between the *trnL* 3´exon and the *trnF* gene in *Cannabis sativa* chloroplasts, as is explained in more details in [1]. Several primer and probe concentration schemes were tested as can be seen in Figure 1 : (1) Purple: 0.5 µmol/L per primer, 0.1 µmol/L probe, (2) Light blue: 0.5 µmol/L per primer, 0.25 µmol/L probe, (3) Rose: 0.25 µmol/L per primer, 0.25 µmol/L probe, and (4) Green: 0.25 µmol/L per primer, 0.1 µmol/L probe.

The real-time PCR reactions were performed in MicroAmp Optical 96-Well Reaction Plates (Applied Biosystems, USA) in a total reaction volume of 25 µL. Each assay comprises 12.5 µL Universal Master Mix (Applied Biosystems, USA), 0.06 µL probe as well as both primers, 7.31 µL in-house bi-distilled water, and 5 µL DNA. The real-time PCR assays were conducted using the ABI 7500 Real-Time PCR System (Applied Biosystems, USA) and the following temperature-time protocol: 2 min at 50 °C, 10 min at 95 °C followed by 45 cycles of 15 s at 95 °C and 1 min at 61 °C. A suitable threshold value of 0.101 was specified by choosing the middle of the linear phase of the amplification graphs.

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RESULTS

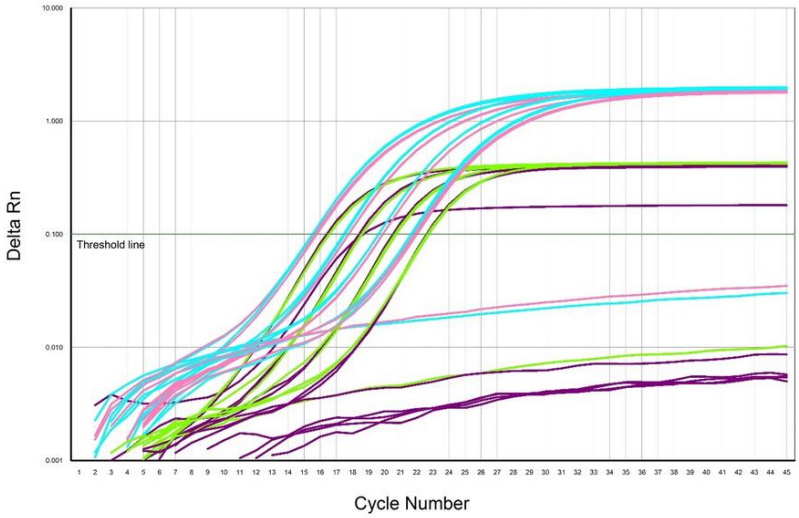

Figure 1: Real-time PCR amplification curves obtained with tested primer and probe concentrations by analysing Monoica *seeds with DNA concentrations of 100 ng/µl, 25 ng/µl, 6.25 ng/µl and 1.5625 ng/µl. (1) Purple: 0.5 µmol/L per primer, 0.1 µmol/L probe. (2) Light blue: 0.5 µmol/L per primer, 0.25 µmol/L probe. (3) Rose: 0.25 µmol/L per primer, 0.25 µmol/L probe. (4) Green: 0.25 µmol/L per primer, 0.1 µmol/L probe. From **[1]**.*

CONCLUSION

Homogenization with the Minilys enables the extraction of high-quality DNA from *Cannabis sativa* seeds, suitable for real-time PCR analysis.

Real-time PCR results show that primer and probe concentration schemes 2 (Light blue, Figure 1) and 3 (Rose, Figure 1) provide the best amplification curves with optimal exponential and plateau phases. For practical reasons, concentration scheme 3, consisting of 0.25 µmol/L per primer and 0.25 µmol/L probe, was chosen. Further results show that this real-time PCR method is suitable for the identification of hemp as a food ingredient with high specificity and a very low limit of detection (0.00031 ng/µL). This method represents a valuable approach to verify and guarantee the presence of hemp and ensure consumer protection.

AGES
Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH

[1] Weck, Sandra, et al. "Development and validation of a real-time PCR assay to detect Cannabis sativa in food." Scientific reports 11.1 (2021): 1-13.