Automated DNA Extraction and Species Analysis from Cheese

Challenge

Analysis of the source species of milk for cheese production.

Solution

Combination of automated nucleic acid extraction, photometry and quantitative real-time PCR (qPCR) for easy-to-use and highly sensitive species analysis for cheese.

Introduction

People are more and more aware of the fact that nutrition has a significant impact on the overall quality of life. Additionally, food scandals of the recent past even sharpened this awareness. For those reasons, customers expect stringent and reliable controls of the ingredients used for food production. Some of these controls (e.g. species identification, allergen identification, halal testing) can be performed using molecular biological methods. The general workflow for molecular biological analysis consists of nucleic acid extraction from sample material followed by quality analysis (e.g. by photometry) and a diagnostic assay mostly based on polymerase chain reaction (PCR). The extraction of nucleic acids from food is challenging due to extensive processing and usage of additives. As soon as nucleic acids are extracted the analysis is dependent on sufficient quantity and quality of DNA and the availability of an assay to detect the parameter of interest, e.g. for species identification.

Herein we describe extraction of deoxyribonucleic acid (DNA) using Analytik Jena's InnuPure C16 *touch* with innuPREP Food DNA Kit-IPC16 (by IST Innuscreen GmbH) specifically developed for extraction of DNA from processed food. After quality control using ScanDrop² and CHIPCUVETTE, extracted DNA is analyzed for the presence of sheep-, goat- and cow-specific genes using innuDETECT Cheese Assay (by IST Innuscreen GmbH) and Analytik Jena's real-time thermal cycler qTOWER³ G.

The workflow for food quality control presented here allows automated extraction of up to 16 food samples in parallel. This enables medium sample throughput with minimum hands-on time and manual operations. Downstream analysis includes parallel detection of DNA quantity and quality with CHIPCUVETTE microfluidic system and species analysis with highly specific and sensitive qPCR.

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Materials and Methods

Samples and reagents

- Two types of commercially available cheese, declared as derived either from goat milk or cow milk
- innuPREP Food DNA Kit-IPC16 (IST Innuscreen GmbH)
- innuDETECT Cheese Assay (IST Innuscreen GmbH)
- 96 Well PCR-Plate (0.2 mL; LP), full-skirted, white (844-70038-S, Analytik Jena GmbH)
- Optical sealing foil (77 × 140 mm), adhesive, transparent, peelable (846-050-258, Analytik Jena GmbH)

Instrumentation

- InnuPure C16 *touch* (845-00020-2, Analytik Jena GmbH)
- BioShake iQ with adapter for 35 × 1.5 mL tubes or 24 × 0.5 mL tubes
- ScanDrop² (844-00204-2, Analytik Jena GmbH) with CHIPCUVETTE (844-70200-0, Analytik Jena GmbH)
- qTOWER³ G (e.g. 844-00554-2, including color modules 1 and 2, Analytik Jena GmbH)

Preparation of DNA from cheese

DNA is extracted with innuPREP Food DNA Kit-IPC16 and InnuPure C16 *touch* according to the following protocol.

200 mg of each cheese sample is transferred into a 1.5 mL reaction tube. Each sample is mixed with 800 µL Lysis Solution CBV and 20 µL proteinase K, followed by vigorous mixing on a vortex. For homogenization, lysis and proteinase K digest the sample is incubated for 1 h at 65 °C shaking with 1000 rpm. Subsequently, solid particles are separated by centrifugation of the lysate for 10 min at 11000 × g. 400 µL of the supernatant is transferred into the Reagent Plate of the innuPREP Food DNA Kit-IPC16. The sample tray carrying the Reagent Plate with samples, disposable pipet tips and elution tubes is put into InnuPure C16 *touch*, followed by start of the protocol in the IPextract software. The end of the extraction protocol has to be confirmed and the extracted DNA can be unloaded. DNA is stored at -20 °C until further use. Each cheese variety is extracted in quadruplicates.

Analysis of DNA quantity and quality

The DNA is quantified using ScanDrop² and CHIPCUVETTE. The ScanDrop family of instruments are polychromatic photometers assessing a whole spectrum within a single and quick detection process. The software for device control is preset to determine the quantity of DNA based on the absorption value of light with a wavelength of 260 nm. The quality is determined by calculation of the ratios of the absorption of light with wavelengths of 260 nm and 280 nm.

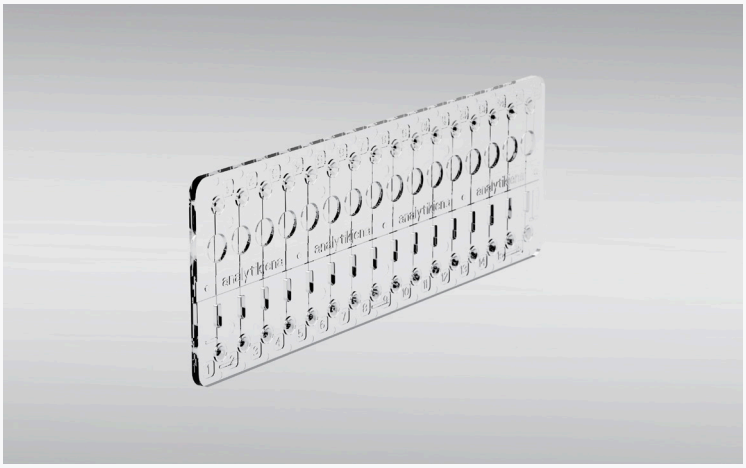

Figure 1: CHIPCUVETTE

Analytik Jena's CHIPCUVETTE (Figure 1) allows analysis of up to 16 samples in parallel (1 reference and 15 samples). Each sample can be detected with pathlengths of 1 mm and 0.1 mm making dilution redundant.

Quantitative real-time PCR analysis of cheese samples

The innuDETECT Cheese Assay contains three individual primer/probe mixes for detection of beef-, sheep- and goat-specific genes. Extracted DNA from each sample was mixed with each of the primer/probe mixes according to Table 1.

Table 1: Pipetting scheme for innuDETECT Cheese Assay.

Component	V [µL]
2 × Mastermix	10
primer/probe mix	3
internal control	1
sample	1
PCR-grade H_2O	ad 20

For non-template control (NTC), PCR-grade water is used instead of sample.
96 well plates and optical sealing films were used as described above. The qTOWER^3 G was programmed as follows (Table 2).

Table 2: Temperature profile for qPCR.

T [°C]	t [s]	Cycles
95	120	
95	10	35
62	45	

The device control software qPCRsoft is used for analysis and data presentation.

Results and Discussion

After extraction of DNA from 200 mg of cheese, concentration and quality were analyzed using photometry (Table 3).

Table 3: Results of photometric analysis of extracted DNA using ScanDrop^2.

Cheese type	Replicate	A_{260}/A_{280}	c [ng/µL]
Goat	1	2.03	9.86
	2	2.02	11.07
	3	2.01	12.56
	4	2.01	12.59
Cow	1	2.07	16.3
	2	1.96	12.78
	3	2.03	12.38
	4	2.01	9.84

The photometric data of Table 3 show that 200 mg cheese is sufficient for the cheese varieties tested to yield DNA for PCR-based downstream applications. Moreover, purity of DNA is good, although a complex and highly processed sample material was used.

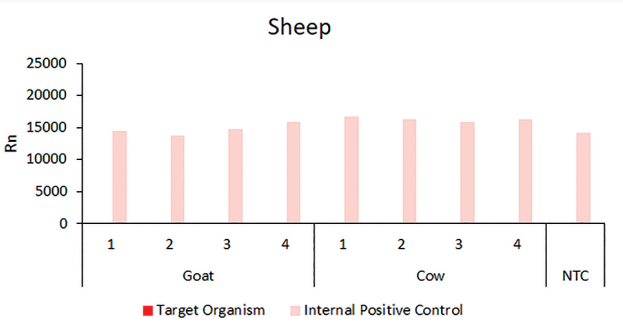

Figure 2: Detection of sheep-specific target gene

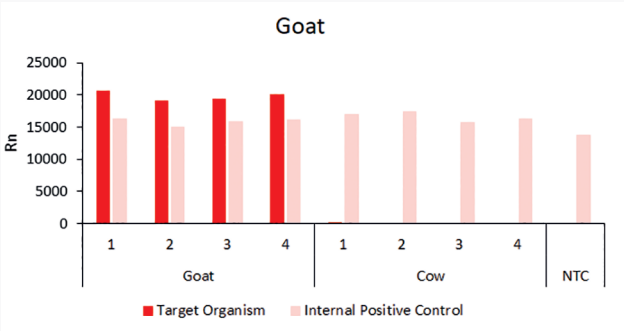

Figure 3: Detection of goat-specific target gene.

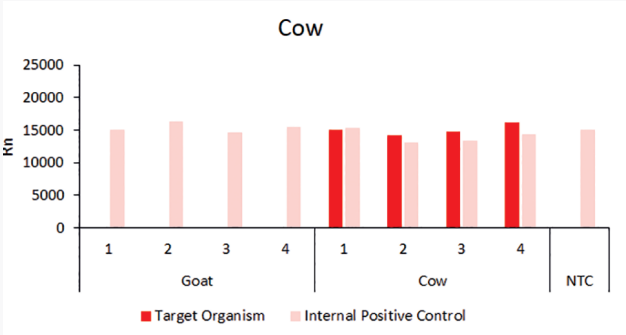

Figure 4: Detection of cow-specific target gene.

qPCR analysis of the samples using the primer/probe mix for sheep-specific genes is negative for all samples of the two cheese varieties (Figure 2). This correlates with expectations as the cheese is declared to be derived from cow and goat milk, respectively. In accordance with this the quadruplicates of the cheese declared as goat cheese are positive for detection of the goat-specific genes and negative for cow-specific genes (Figure 3) while the cheese declared to be derived from cow milk is negative for goat-specific genes and positive for cow-specific genes (Figure 4). False negative results can be excluded as Internal Positive Control is detected in DNA of all extracted samples.

Conclusion

The results show that the workflow of DNA extraction using Analytik Jena's InnuPure C16 *touch* with innuPREP Food DNA Kit-IPC16 in combination with innuDETECT Cheese Assay and Analytik Jena's qTOWER^3 allows safe and reliable determination of the origin of milk in cheeses.

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