Challenge

Extraction of high quality DNA from various food samples for PCR-based downstream applications

Solution

Extraction of high quality DNA using the InnuPure extraction devices and innuPREP kits. Extracted DNA can readily be used for assays based on quantitative real-time PCR (qRT-PCR).

Automated Extraction of DNA from Various Food Samples

Introduction

Quality food and its impact on health is becoming more important to costumers. This aspect together with food scandals of the recent past push the food industry to make their products more transparent. One important issue is the origin of raw materials. This point touches topics like the use of materials derived from genetically modified organisms as well as the use of the correct materials indicated on the list of ingredients. An effective method to investigate the origin of materials and genetic background of ingredients in processed food are technologies based on Polymerase Chain Reaction (PCR), as these methods are very sensitive. In order to use PCR-based technologies for the analysis of food, a certain amount and quality of DNA is required. Some sample materials (e.g. plant samples, processed food) contain significant levels of inhibitors, which inhibit PCR-based downstream applications and therefore interfere with analysis.

In the experiments described below, DNA was extracted from northern shrimp, hazelnut and two different kinds of cheese. DNA was extracted using innuPREP DNA Kit-IPC16 and innuPREP Food DNA Kit-IPC16. Extraction was performed with InnuPure C16/C16 *touch*. After homogenization (optional) and lysis, lysed samples were transferred in the Reagent Plastics of the kits. Reagent Plastics of the kits are pre-filled with all required buffers and magnetic particles for DNA extraction.

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The Reagent Plastics were loaded in the InnuPure C16/C16 *touch* followed by fully automated extraction. Following extraction, DNA quality was analyzed using gel electrophoresis, spectrophotometry and quantitative real-time PCR (qRT-PCR) for species analysis.

The high pre-processing state of the kits (pre-filled, sealed buffer reservoirs) and automated purification reduces the hands-on time of the customer to a minimum. Besides the benefit of reduced time consumption, pre-processing also reduces the risk of human errors and loss of valuable samples. Analysis of extracted DNA by agarose gel electrophoresis, spectrophotometry and qRT-PCR analysis shows that DNA extracted with devices and kits from Analytik Jena can be readily used for relevant downstream applications.

Materials and Methods

Frozen northern shrimps (50 mg) were homogenized in 200 µL H_2O for 15 s using SpeedMill PLUS and Lysis Tubes P (Analytik Jena). Homogenates were mixed with 200 µL Lysis Solution CBV and 20 µL Proteinase K and incubated at 50 °C shaking with 600 rpm for 60 min. 400 µL of the lysate were transferred to the Reagent Plastic of the innuPREP DNA Kit-IPC16 followed by extraction with InnuPure C16.

100 mg and 200 mg ground hazelnut was resuspended in 800 µL Lysis Solution CBV and 20 µL Proteinase K. The samples were lysed for 60 min at 65 °C. After lysis, 400 µL supernatant were used for extraction with the innuPREP Food DNA Kit-IPC16 and InnuPure C16.

DNA was extracted from two varieties of commercially available cheese. Cheeses were declared to be derived from goat and cow milk, respectively. Extraction was performed in quadruplicates using InnuPure C16 *touch* and innuPREP Food DNA Kit-IPC16. 200 mg of cheese were used for each replicate. Elution volume was set to 100 µL. Following extraction, extracts were analyzed by photometry using ScanDrop 250 and qRT-PCR. For qRT-PCR qTOWER^3 and innuDETECT Cheese Assay were used. The analysis was performed with Endpoint method provided by qPCRsoft.

Samples and Reagents

- innuPREP DNA Kit-IPC16
- innuPREP Food DNA Kit-IPC16
- Lysis Tubes P
- innuDETECT Cheese Assay
- Commercially available kit for hazel analysis

Instrumentation

- InnuPure C16
- InnuPure C16 *touch*
- SpeedMill PLUS
- BioShake iQ
- ScanDrop 250
- Standard equipment for agarose gel electrophoresis and gel documentation
- qTOWER^3 G

Results and Discussion

After extraction of DNA from frozen shrimp samples, DNA quality and integrity was assessed by spectrophotometrical analysis with ScanDrop 250 and agarose gel electrophoresis.

Table 1: Spectrophotometrical analysis of DNA extracted from shrimp samples.

Sample number	A_{260}/A_{280}	c [ng/µL]	c_{mean}±SD [ng/µL]
1	2.32	23.64	22.1±1.8
2	1.96	21.26	
3	2.25	23.51	
4	1.87	19.94	

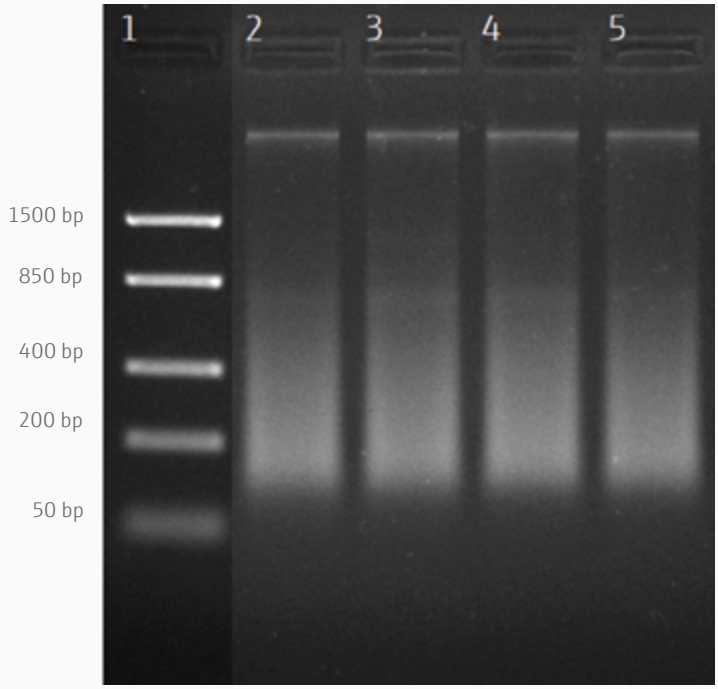

Figure 1: Agarose gel electrophoresis of DNA extracted from frozen shrimp samples. Lane 1: DNA ladder; lanes 2–5: Samples 1–4.

The innuPREP DNA Kit-IPC16 in combination with the InnuPure C16 enables the automated DNA extraction from shrimps with minimal hands-on time. As a result of extraction, pure DNA with A_{260}/A_{280} ratios ranging from 1.87 to 2.32 is obtained. Mean yields of 22.1 ng/µL are sufficient for PCR-based downstream applications. Agarose gel analysis shows that a part of the DNA is degraded which appears as a smear in lanes 2–5 of Figure 1. DNA degradation is a natural process which starts shortly after the death of the animals and stops with freezing. Besides degraded DNA, quite some high molecular weight DNA is visible as a distinct band with a size larger than 1500 bp.

After DNA extraction from 100 mg and 200 mg ground hazelnut with the innuPREP Food DNA Kit-IPC16, extracts were used for qRT-PCR-based detection of hazelnut DNA. In order to detect inhibitory effects of substances contained in the extracted DNA, aliquots of the extract were diluted 1:10 followed by amplification of 1 µL undiluted and 1:10 diluted extract.

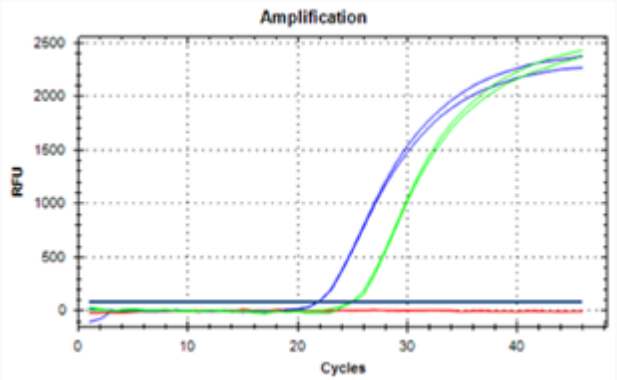

Figure 2: DNA extracted from 100 mg ground hazelnut, amplified and detected with qRT-PCR in duplicates (blue curves undiluted, green curves 1:10 dilution).

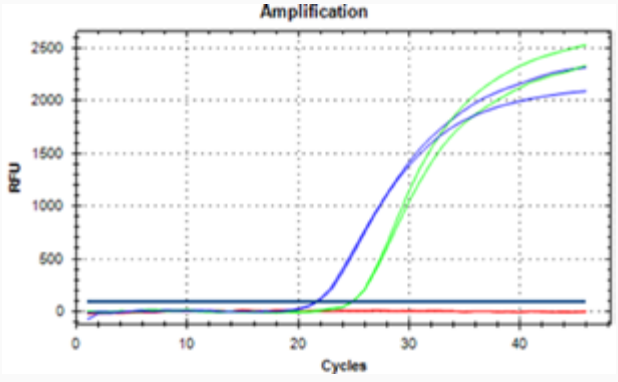

Figure 3: DNA extracted from 200 mg ground hazelnut, amplified and detected with qRT-PCR in duplicates (blue curves undiluted, green curves 1:10 dilution).

Figures 2 and 3 show that DNA extraction from both, 100 mg and 200 mg ground hazelnut, is possible and yields DNA which can readily be used for qRT-PCR. Sample materials with high contents of lipids are often challenging with respect to extraction as well as to contamination of the extracted DNA with PCR inhibitors. For the extraction of DNA from hazelnut, PCR inhibition can be excluded as ten-fold dilution of the sample. It results in a shift of the Ct value of around 3.3 cycles which is the case for extracts from 100 mg and 200 mg, respectively. DNA was extracted from 200 mg cheese as described above. Following extraction, extracts were analyzed by photometry using ScanDrop 250 and qRT-PCR. qTOWER^3 and innuDETECT Cheese Assay were used for qRT-PCR. Analysis was performed with Endpoint method provided by qPCRsoft.

Table 2: Photometric analysis of nucleic acid extracted from cheese samples.

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

Figure 4: Detection of sheep-specific target gene.

Figure 5: Detection of goat-specific target gene.

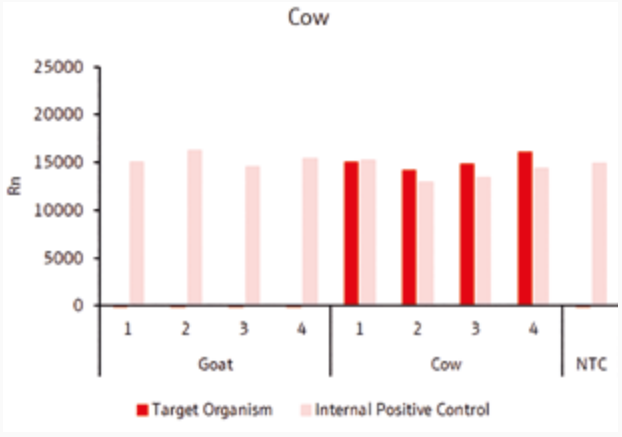

Figure 6: Detection of cow-specific target gene.

Results show that automated extraction using InnuPure C16 *touch* in combination with the innuPREP Food DNA Kit-IPC16 can be used to prepare DNA from cheese. Quality and quantity are sufficient for subsequent qRT-PCR application as depicted in Figure 4-6. Analyzing 200 mg starting material leads to eluates with concentrations ranging from 9-16 ng/µL. Moreover, proteins are completely removed using the innuPREP Food DNA Kit-IPC16 as indicated by ratios A_{260}/A_{280} in Table 2. The samples derived from milk with known origin were subjected to qRT-PCR analysis of sheep-, goat- and cow-specific genes. In all cases, internal positive control (light red) was detected. However, goat-specific genes were only detected in cheese declared to be derived from goat milk while cow-specific genes were only detected in cheese declared to be derived from cow milk. None of the samples was positive for sheep-specific genes.

Conclusion

In summary, the results show that combination of InnuPure C16 *touch* and DNA extraction kits for this device can be used for DNA extraction from a multitude of samples related to food industry. DNA extracted with this system has optimum quality. Most important, DNA extracted with InnuPure C16 *touch* and the relevant kits can readily be used for qRT-PCR.

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