Extraction of DNA from Cheese Using ProteoSolve-SB and Pressure Cycling Technology (PCT)

Introduction

Some foods, such as cheese, are made by fermentation with microorganisms including yeast, mold and bacteria. To obtain the desired type and quality of cheese, it is essential to introduce the correct microorganisms in the proper ratio during the manufacturing process. Thus it is desirable to be able to determine which organisms are present. In addition, it is sometimes necessary to confirm the milk source in order to ensure authenticity and proper manufacturing of the cheese. This can be accomplished by isolating total DNA from the finished product and then probing for the presence of DNA from either the microorganism of interest, or the mammalian milk source. However, extraction of DNA from samples such as cheese is often hampered by high levels of lipid and protein and the relatively low levels of DNA. Typically, DNA is isolated from cheese by first digesting the proteins with Proteinase K and then isolating DNA from the resulting lysate. This is a lengthy procedure as homogenization and digestion may require several hours [1], or even overnight incubation [2]. Thus, sample disruption and digestion are both time consuming and inefficient steps in isolation of DNA from cheese. Here we describe a method for the simultaneous extraction of DNA, RNA, proteins and lipids from various cheeses by using PBI's ProteoSolve-SB Kit and pressure cycling technology (PCT).

ProteoSolve-SB Kit

The ProteoSolve-SB kit (Pressure BioSciences) is designed for concurrent, detergent-free extraction and fractionation of proteins, lipids and nucleic acids from cells, tissues or complex matrices. Following extraction, the sample is separated by centrifugation into three fractions - a lipid-containing upper phase, a protein-containing lower phase, and an insoluble fraction (pellet and interface), which contains the DNA and RNA - as well as a small amount of protein. The DNA and/or RNA can be isolated from the residual solid fraction. During cycles of high hydrostatic pressure and rapid de-pressurization, cells are ruptured and dissolved in the solvent mixture, which becomes nearly homogenous under pressure. Upon de-pressurization, solvent phases separate and fractionate the sample constituents according to their inherent solubility [3].

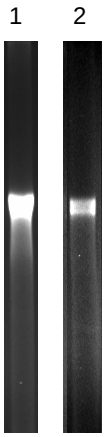

Figure 1. Examples of DNA isolated from Brie (Lane 1) and Camembert (Lane 2) Cheeses. DNA was visualized on agarose minigels stained with ethidium bromide.

PCT Sample Preparation System (PCT SPS)

Pressure Cycling Technology Sample Preparation System (PCT SPS) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. The PCT SPS can be used to disrupt tissues, cells, and cellular structures to extract lipids, proteins and nucleic acids [4]. The PCT SPS uses a small, semi-automated bench top instrument (Barocycler NEP3229 or the NEP2320) in combination with single-use sample processing containers called FT500 PULSE Tubes (Pressure BioSciences).

Materials and Methods

DNA was extracted from French Bleu, American Camembert, French Brie and Italian Pecorino cheese. Cheeses were stored at 4˚C prior to extraction. Each type of cheese (0.3-0.4 g) was loaded into FT500 PULSE Tubes. The Ram was then inserted as far as possible, pushing the cheese up to or through the lysis disc in the PULSE Tubes. (For cheeses expected to contain little DNA, 1 gram of cheese was split into three PULSE Tubes for processing, and the resulting extracts were pooled after PCT.) ProteoSolve-SB Reagent A (1.1-1.2 mL) was added to each PULSE Tube. Due to the high lipid content of the cheeses tested, Reagent B from the ProteoSolve-SB was not necessary and was omitted. Samples were vortexed for 10 s prior to PCT. For the Pecorino sample, *The PCT Shredder* (Pressure BioSciences) [5] was used to facilitate extraction. In this case, Pecorino was homogenized by shredding for 20 sec prior to PCT. All samples were subjected to 30 cycles of PCT for 20 sec at 35,000 psi followed by 10 sec at atmospheric pressure at ambient temperature. After PCT, the extracts were transferred to 2 mL microcentrifuge tubes and centrifuged at 12,000-14,000 x g for 15 min to separate the DNA-containing insoluble material from the dissolved lipids and proteins. Most of the liquid was then removed leaving ~0.1-0.2 mL behind with the pellet. To help break up the pellet, the pellet and residual material was then mixed by vortex. The partially purified material was then re-extracted by adding 1.2 mL of fresh Reagent A to the mixture and transferring the suspension back to the original PULSE Tube. The sample was then subjected a second round of PCT and subsequent centrifugation, as described above, to remove additional proteins and further enrich for DNA. After the second centrifugation, the protein and lipid-containing liquid was removed and the insoluble material was used for DNA isolation. Pecorino samples that had been split into multiple PULSE Tubes were pooled as follows: liquid extract was removed leaving

each of the 3 pellets in ~0.1-0.2 mL of solvent. The pellets were vortexed and combined into one 2 mL centrifuge tube. The pooled material was then centrifuged at 12,000-14,000 x g for 10 min and as much residual liquid as possible was removed. Pellets with more than ~50 µL of residual liquid were then dried briefly in a SpeedVac to minimize carry-over of solvent. DNA may be purified from the final pellet by any of several standard protocols such as DNAzol (Invitrogen) or the DNeasy Blood and Tissue Kit (Qiagen). With the DNeasy method, the standard tissue protocol was used (180 µL of buffer ATL and 20 µL of Proteinase K per pellet, incubated at 56˚C for at least an hour). In order not to clog the DNeasy column with the residual solid material after the Proteinase K digestion, the lysate was centrifuged for 2-3 min at low speed (~1000 x g) to remove the insoluble material. When the DNAzol extraction method was used, 0.9 mL DNAzol per final pellet was found to be sufficient. Since the pellet material did not always fully dissolve in the DNAzol and could contaminate the DNA preparation, a chloroform extraction was performed (as described in the Invitrogen plant DNAzol protocol). Following DNA isolation, DNA recovery was measured by Qubit assay using the Quant-iT dsDNA BR kit (Invitrogen). DNA was visualized by agarose gel electrophoresis using the Reliant FastLane Gel System (Lonza).

Results and Discussion

DNA was isolated from four different types of cheese (Table 1). Soft cheeses that are known to contain relatively high amounts of flora, such as Bleu, Brie and Camembert, yielded ~2-4 µg of purified DNA per gram of cheese. Pecorino, a ewe's milk hard cheese with little visible flora, yielded ~0.3 µg of DNA per gram of cheese. Isolated DNA was visualized by agarose gel electrophoresis. The presence of high molecular weight genomic DNA (Figure 1) confirmed that the DNA was not severely sheered or degraded during the isolation procedure. These results show that the ProteoSolve-SB kit in combination with PCT is an effective tool for isolating intact DNA from a variety of hard and soft cheeses.

Cheese	DNA Recovery
French Bleu	3.8 µg
French Brie	2.0 µg
American Camembert	4.1 µg
Italian Pecorino	0.3 µg

Table1. DNA Extracted from Four Types of Cheeses. Recovery is expressed in µg DNA per gram of cheese.

DNA extraction from samples such cheese may be hampered by the presence of high amounts of protein and lipid, and the relatively low amounts of DNA. The unique chemistry of the ProteoSolve-SB kit in combination with the efficient sample disruption made possible by PCT, permits efficient extraction of DNA and removal of the protein and lipid components of the cheese, leaving a DNA-enriched pellet from which high quality DNA may be isolated using commonly available and well characterized kits or reagents. DNA prepared in this method is suitable for analysis by specific probes or by DNA sequencing.

References

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