1. Introduction

Pressure Cycling Technology (PCT) uses alternating cycles of high and low hydrostatic pressure to effectively induce the lysis of cells and tissues in preparation for two-dimensional electrophoresis (2DGE) and other analytical or preparative methods. Rapid cycling between high and low pressure is more disruptive than high pressure alone, as evidenced by the increased protein yields from Saccharomyces cerevisiae correlating to the number of pressure cycles rather than the total elapsed time at high pressure [1]. Similarly, proteins of increasing molecular mass correlating to the number of pressure cycles were released from the chitin exoskeletons of the arthropod Daphnia pulex [2].

Previously, Geiser et al. [3] reported the PCT release 37% more protein from the nematode Caenorhabditis elegans than sonication. From gram-negative bacteria, PCT reportedly yielded 14.2% more protein from Escherichia coli than bead mill [4] and 17.1% more protein from Rhodopsseudomonas palustris than enzymatic lysis with lysozyme [5].

For plant tissues, PCT extracted more protein from several components of the Streptella regiae inflorescence than a centrifugal homogenizer [2]. For mammalian tissues, PCT also isolated more protein from liver, including unique proteins which were not isolated in Polytron or douncer homogenates [6]. From adipose tissue, PCT extracted more protein than pulverization under liquid nitrogen and detergent extraction of the triturate.

2. Materials and Methods

2.1 Pressure Cycling Technology

PCT uses alternating cycles of high and low pressure to induce cell lysis. Cell suspensions or tissues are placed in specially designed single-use PULSE Tubes (Figure 1) and are subjected to alternating cycles of high and ambient pressure in a pressure-generating instrument (Barocycler NEP2209). Maximum and minimum pressures (0-35,000 psi), the time sustained at each pressure level, and the number of cycles is defined using programmable logic controller interface. The Barocycler pressure chambers is temperature controlled using a peripheral circulating water bath. Safety features in the PCT system design significantly reduce risk of exposure to the researcher to pathogens. Recently, two researchers from the U.S. Center for Disease Control were infected with West Nile Virus when tubing ruptured during bead mill operation [4].

2.2 Sample Preparation

Unless otherwise specified, samples were processed in ProteoSOLVE CE Lysis Reagent (Pressure Biosciences, West Bridgewater, MA, USA) supplemented with 100 mM DTT and protease inhibitor cocktail P-2714 (Sigma-Aldrich Chemicals, St. Louis, MO, USA). For PCT, samples were typically subjected to 20 pressure cycles; each cycle consisting of 20 seconds at 35,000 psi followed by 20 seconds at ambient pressure. Following PCT, the samples were centrifuged for 10 minutes at 25,000 RPM and 100,000 g, of the sample was transferred to an Ultrafree Centrifugal Filtration Device (Millipore, Danvers, MA, USA) and centrifuged until a retentate volume of 100 ul was obtained. Four hundred microfilters of ion-exchanged ProteoSOLVE IEF Reagent was added (reducing the DTT concentration to 20 mM) and the sample was alkylated for two hours following the addition of 40 mM acrylamide and 40 mM Tris. The alkylation reaction was terminated by resuming centrifugation in the ultrafiltration device [5].

2.3 SDS PAGE, IEF, and 2DGE

IEF and 2DGE was performed as described previously by Smejkal et al. [5]. Extracts from adipose tissues were analyzed by SDS PAGE on NuPAGE Novex 4-12% Bis-Tris gels using MOPS-SDS running buffer (Invitrogen, Carlsbad, CA, USA).

3. Results and Discussion

3.1 PCT vs. Enzymatic Lysis

PCT used in combination with the chaotropic ProteoSOLVE Lysis Reagent yielded 17.1% more protein than enzymatic lysis with recombinant lysozyme from the gram-negative bacterium Rhodopsseudomonas palustris (Figure 2). PCT isolated several putative membrane proteins from P. palustre, which were not isolated by enzymatic lysis, which appears biased for cytosolic proteins.