

## Increased Protein Yields from Coniferous Plants Using *The PCT Shredder*<sup>™</sup> and Pressure Cycling Technology (PCT)

### Introduction

The plant proteome provides the opportunity to monitor post-translational response to environmental influences such as pollution [1], insect infestation [2], or plant diseases [3]. Comprehensive proteomic analyses require reliable extraction methods that isolate proteins reproducibly and without bias. Sample preparation of plant tissues is particularly challenging due to the nature of cell walls, which make it difficult to quantitatively extract analytes, the relatively low cellular content of proteins in some plant tissues, or the abundance of lignin, tannin, and other polyphenols that can interfere with protein analyses. The extraction of proteins from pine needles and other coniferous tissues is particularly challenging, as noted by Cai-Yun *et al.* [4], and may be further complicated in these species by their high content of terpene resins. Here we describe a system for the efficient extraction of proteins from two conifers, *Pinus strobus* (Eastern White Pine) and *Thuja standishii* (Japanese Arborvitae). Initial disruption of plant tissue with *The PCT Shredder* followed by protein extraction using pressure cycling technology (PCT) is carried out in the presence of various extraction buffers in the same processing container (Shredder PULSE Tube). This method of extraction is safe, convenient and efficient. In combination, *The PCT Shredder* and PCT extracted up to 2-3 times more protein from pine needles than the BioMasher<sup>™</sup> centrifugal homogenization device (See Table 1). Data also show that nondenaturing and strong denaturing extraction buffers can effectively be used in combination with *The PCT Shredder* and PCT to extract proteins from both *P. strobus* and *T. standishii* for analysis.

### Pressure Cycling Technology (PCT)

In the Pressure Cycling Technology Sample Preparation System (PCT SPS) hydrostatic pressure is rapidly cycled between ambient and ultra high levels (35,000 psi) to control biomolecular interactions [5]. High hydrostatic pressure acts preferentially on the compressible components of the sample, such as cell membranes, resulting in cell lysis and the release of intracellular contents. The PCT SPS can be used to disrupt plant and animal tissues, cells, cellular structures and microbes to extract nucleic acids, proteins and lipids. The system is comprised of a small, semi-automated bench-top instrument (Barocycler NEP3229 or the NEP2320) used in combination with single-use sample processing containers (PULSE Tubes). PCT in the presence of suitable extraction reagents, such as nondenaturing or strong denaturing buffers results in isolation of proteins for analysis.

Table 1. Comparison of PCT Shredder and the BioMasher centrifugal homogenizer for the extraction of protein from pine needles

description	actual sample mass (mg)	protein (mg/mL)	tannins (405 nm)
BioMasher	202.0 ± 1.0	0.437 ± 0.048	0.163 ± 0.041
PCT Shredder only	198	0.737	0.391
PCT Shredder plus PCT	200.5 ± 4.5	1.302 ± 0.021	0.530 ± 0.092

*Proteins extracted from pine needles in Reagent D from the ProteoSOLVE SB Kit.*

### *The PCT Shredder*

*The PCT Shredder* is designed to physically disrupt and enhance extraction of tough, fibrous and other difficult-to-disrupt biological materials such as certain plant and animal tissues [6]. *The PCT Shredder* is used to rapidly grind the sample directly in a specially designed Shredder PULSE Tube to increase the tissue surface area and to improve cell lysis prior to treatment by PCT for extraction of nucleic acids, proteins, lipids and other cellular contents. Since shredding and PCT are done in the same tube, loss of sample or the likelihood of cross contamination is significantly reduced as compared to other processing methods.

### Materials and Methods

Needles from *P. strobus* and leaves from *T. standishii* were harvested and processed within one hour of collection. The plant tissue were then coarsely chopped to 2-3 mm length and weighed into tared Shredder PULSE Tubes or FT500 PULSE Tubes in 50, 200, or 350 mg aliquots to which 1350 uL of nondenaturing buffer (ProteoSolve NATIVE or NATIVE Plus) or strongly denaturing buffer (ProteoSolve CE PrEP Kit or ProteoSolve SB Kit) was added. All reagents were supplemented with protease inhibitors (Sigma-Aldrich, St. Louis, MO). PCT was performed with and without using *The PCT Shredder* to demonstrate the efficacy of extraction by each method, as well as to demonstrate the cumulative effect of using chemistry, *The PCT Shredder* and PCT in combination.

For comparison, 50 or 200 mg of conifer tissue was processed using a BioMasher centrifugal homogenizer (Cartagen Molecular, San Carlos, CA) by grinding against the porous polyethylene membrane of the BioMasher insert with the homogenizer bar. Two separate aliquots of 700 uL of buffer were added and the combined homogenate was collected by centrifugation. Coniferous biomasses larger than 200 mg could not be accommodated in the small insert of the BioMasher<sup>™</sup>.

## Results and Discussion

The PCT Shredder yielded nearly twice the protein from 200 mg of *P. strobus* needles than the BioMasher centrifugal homogenization device (Table 1) and three times the protein than the BioMasher when followed by PCT for 40 cycles at 35,000 maximum pressure. From 50 mg samples, The PCT Shredder and the BioMasher yielded similar amounts of protein suggesting that the BioMasher™ was only effective for processing relatively small amounts of sample.

In other experiments, the effectiveness of various buffers in combination with The PCT Shredder and PCT were evaluated. ProteoSolve NATIVE and NATIVE Plus buffers were designed to extract proteins under relatively mild conditions. These buffers are required when preservation of the native conformation and biological activity of proteins prohibits the use of chaotropes or detergents. ProteoSolve NATIVE Plus contains a mild nondenaturing surfactant to increase the solubility of hydrophobic protein; its use resulted in a 56% increase in protein yields from the more resinous *T. standishii* compared to the other buffers evaluated (See Table 2).

Table 3: Comparative protein yields from *P. strobus* needles using the PCT Shredder and nondenaturing or strongly denaturing reagents

method	extraction reagent	protein assay (mg/mL)	tannins (405 nm)
PCT Shredder only	ProteoSolve NATIVE	0.366 ± 0.007	0.121
PCT only		0.583 ± 0.068	0.162
PCT Shredder plus PCT		0.896 ± 0.008	0.215 ± 0.007
PCT Shredder only	NATIVE Plus	0.270 ± 0.012	0.165
PCT only		0.544 ± 0.018	0.204
PCT Shredder plus PCT		0.603 ± 0.021	0.291 ± 0.019
PCT Shredder only	ProteoSolve CE	5.085 ± 0.224	0.271
PCT only		3.712 ± 0.330	1.087
PCT Shredder plus PCT		5.461 ± 0.328	1.379 ± 0.066

From 350.0 ± 1.0 mg pine needles

## Conclusions

The synergy between mechanical disruption of The PCT Shredder and the use of high hydrostatic pressure is an effective method to extract proteins from coniferous needles and leaves from *P. strobus* and *T. standishii*. Comparisons show that The PCT Shredder used in combination with PCT yielded more total protein than either method individually. Relatively high protein yields were obtained under nondenaturing conditions using the NATIVE and NATIVE Plus buffers, which is desirable to preserve protein interactions and activity. However, even higher protein yields may be obtained using reagents such as ProteoSolve CE, although biological activity may be lost, since chaotropes reduce many proteins to their primary structure.

## References

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Table 2: Comparative protein yields from *T. standishii* leaves using the PCT Shredder and nondenaturing or strongly denaturing reagents

method	extraction reagent	protein assay (mg/mL)	tannins (405 nm)
PCT Shredder only	ProteoSolve NATIVE	0.133 ± 0.043	0.843
PCT only		0.270 ± 0.088	0.505
PCT Shredder plus PCT		0.410 ± 0.029	1.147 ± 0.228
PCT Shredder only	NATIVE Plus	0.452 ± 0.048	1.153
PCT only		0.369	0.386
PCT Shredder plus PCT		0.640 ± 0.072	1.194 ± 0.333
PCT Shredder only	ProteoSolve CE	1.657 ± 0.302	0.814
PCT only		1.855 ± 0.041	1.178
PCT Shredder plus PCT		2.456 ± 0.150	2.111 ± 0.166

From 350.4 ± 0.7 mg *T. Standishii* leaves.

The use of chaotropes, detergents, and reducing agents effectively dissociates non-covalent protein interactions and covalent S-S linkages resulting in significantly higher protein recoveries. The ProteoSolve CE Reagent from the CE PreP Kit was specifically designed for maximal protein yields from recalcitrant samples in applications where the preservation of biological activity is not required [7]. ProteoSolve CE yielded an order of magnitude more protein from pine needles than the milder, nondenaturing buffers (See Table 3).