Enzyme-Assisted Protein Isolation from Alfalfa Leaves

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RATIONALE & OBJECTIVES

- Alfalfa leaves are one of the most important raw leaf protein sources due to the high crude protein content and balanced amino acid composition ratio. Producing food grade leaf proteins requires development of a suitable extraction process that results in high extraction efficiency and is mild enough to preserve amino acids.
- This work investigates the use of plant cell-wall degrading enzymes (cellulases, hemicellulases, and pectinases) in assisting the extraction of proteins from alfalfa leaves and to extend the use of alfalfa leaf proteins as a source of bioactive peptides.

STUDY DESCRIPTION

Alfalfa leaves:

Fresh alfalfa leaves of the "HarvXtra" variety, harvested from Mann Valley Farm at UW-River Falls.

Enzymes used:

Cellic CTec2 (C), Viscozyme L (V), and Pectinex Ultra SP-L (P) enzymes (Novozymes Corp., Copenhagen, Denmark) were used in various combinations to degrade cell-wall structure of alfalfa leaves.

Enzyme-assisted protein extraction:

Mixtures of enzymes and alfalfa slurry incubated for 24 hrs at 50°C, 150 rpm, followed by pH adjustment to 9.0 using NaOH to extract alfalfa leaf proteins. The extracted proteins were precipitated and recovered by acid coagulation.

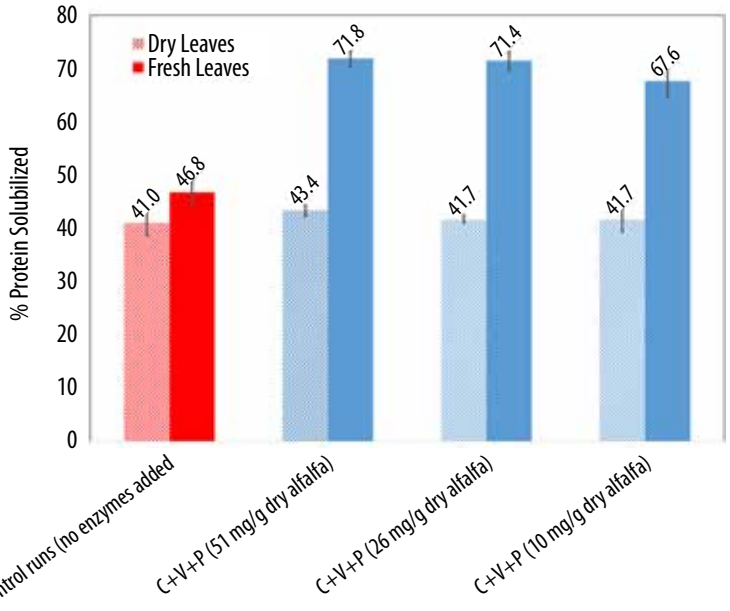
Analysis:

Samples from enzymatic extraction were analyzed for soluble proteins by using DC protein assay. Acid-precipitated proteins were analyzed for the contents of amino acids and crude proteins. Peptides obtained from the alfalfa leaf proteins were tested for antioxidative activity.

RESULTS

- Figure 1: Protein extractability was enhanced by roughly 30% for fresh leaves than for dried leaves with enzymes. Drying leaves has a negative impact on the protein extraction efficiency when enzymes are used. The results indicate that the use of fresh leaves is desirable in the enzyme-assisted protein extraction.

Figure 1. Effect of cell-wall degrading enzymes on protein extraction of dry vs. fresh alfalfa leaves. Enzymes added: Cellic CTec 2 (C); Viscozyme L (V); Pectinex Ultra SP-L (P). Control: no enzymes were added.



- Figure 2: The enzyme-assisted extraction of alfalfa proteins significantly improved % protein extraction (65%-75%, depending on the enzymes used) than the typical pressing method reported in the literature. Cellic CTec2 (C) has no significant impact on the protein extraction from alfalfa leaves, while the other two enzyme blends (V, P) enhance protein extraction by at least 12-23% even at a low enzyme loading. A single enzyme blend of Viscozyme L at >10 mg/g dry alfalfa or Pectinex Ultra SPL at >1.5 mg/g dry alfalfa, or a dual enzyme mixture comprising Viscozyme and Pectinex at minimum 6 mg/g dry alfalfa was determined as the optimum enzyme dose for protein extraction from alfalfa leaves.

Figure 2. Effect of individual enzyme blend (**A**) and multiple enzyme blends (**B**) on protein extraction of fresh alfalfa leaves. Enzymes added: Cellic CTec 2 (C); Viscozyme L (V); Pectinex Ultra SP-L (P). Control: no enzymes were added.

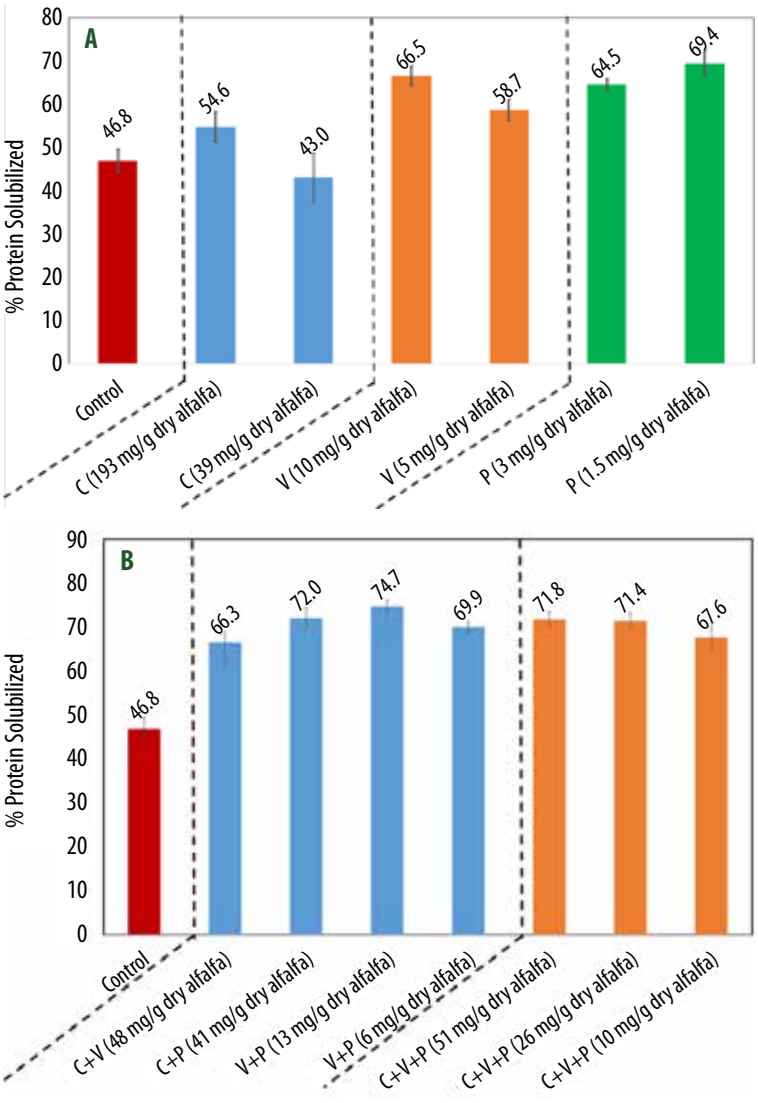
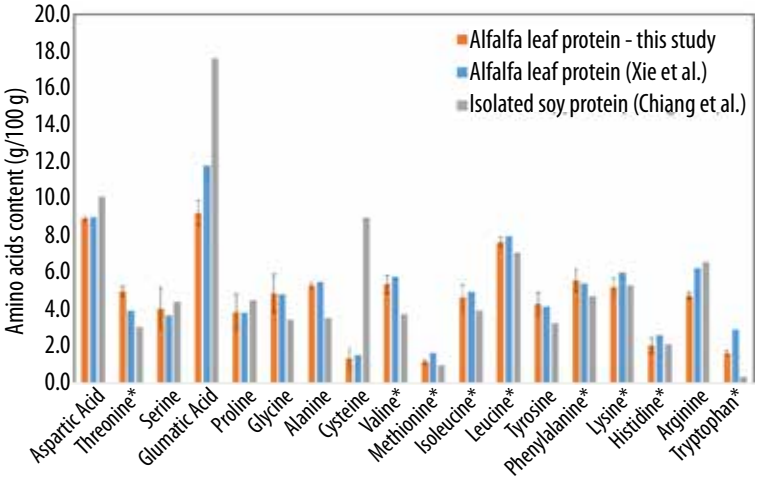
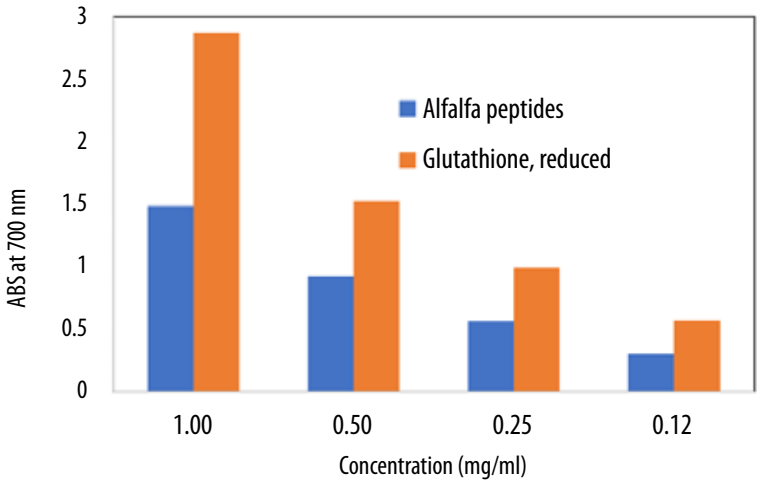


Figure 3. Amino acid compositions of alfalfa leaf proteins and isolated soy protein. *Essential amino acids.



- Figure 3: The protein obtained with enzyme-assisted alkaline extraction and acid coagulation contained 30.1% total amino acids (by dry wt) while the protein obtained without the use of enzymes (control) contained 22.6% total amino acids. The leaf protein composition was compared to that of isolated soy protein. Except for glutamic acid and cysteine, nearly all amino acids contents were close to those reported for isolated soybean protein. Several essential amino acids (Thr, Val, Ile, Leu, Phe, and Trp) were compared favorably to those in isolated soy protein.
- Figure 4: Alfalfa leaf protein was hydrolyzed to peptides using Alcalase (a commercial protease). The peptides were analyzed for their antioxidative activities. Roughly two times higher concentration of alfalfa peptides than that of GSH exhibits an equivalent level of reducing power as GSH. The presence of antioxidative free amino acids (Trp, Tyr, Met, Cys, His, Phe and Pro) in alfalfa leaf protein (see Figure 3) can promote the antioxidant activities of alfalfa protein hydrolysate.

Figure 4. Reducing power of alfalfa peptides vs. reduced glutathione (GSH). Values are the means of duplicate measurements.



CONCLUSIONS

- The use of cell-wall degrading enzymes (Viscozyme L, Pectinex Ultra SP-L) enhances the protein extraction of alfalfa leaves by a factor of 1.4-1.6 as compared to a non-enzymatic extraction process.
- Alfalfa leaf protein has desirable nutritional quality and could be a good source of protein-rich additives and dietary antioxidant supplements for livestock animals and human consumption.

Alfalfa
U.S. Alfalfa Farmer Research Initiative

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