Enzyme-Assisted Protein Isolation from Alfalfa Leaves
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Project Award: $24,183

Justification:
- Green leaves are recognized as one of the largest renewable sources of high quality protein for human and animal consumption. Extraction of proteins from leaves is technically and economically more attractive than from whole plants due to the relatively high protein and low lignin contents of leaves.

Alfalfa leaves are one of the most important raw leaf protein sources due to the high crude protein content (260 g/kg dry matter or 2600 kg of protein per ha) and balanced amino acid composition ratio which is consistent with the Food and Agriculture Organization (FAO)'s recommended adult amino acid profile (Hojilla-Evangelista et al., 2017; Zhang et al. 2017). However, application of alfalfa proteins for human consumption has been limited by undesirable qualities, such as color, taste, and texture.

Alfalfa leaf proteins consist of an equal fraction of "white" and "green" proteins. (Zhang et al. 2017). The white protein fraction has the benefit of high digestibility in humans, desirable functional properties such as excellent emulsification, heat stability and good water solubility. The green protein fraction lacks these positive qualities and also has negative sensory properties (Bickoff et al., 1975; Chen and Qiu, 2003). Discarding green insoluble proteins would improve the sensory properties of alfalfa protein isolate for human consumption but at the cost of losing nearly half of the potential protein yield. Such limitations can be overcome by applying enzymatic hydrolysis to produce peptides that are easier to digest with improved solubility and sensory properties.

Enzymatic hydrolysis of alfalfa leaf proteins (both white and green isolates) can also produce high-value antioxidative peptides. Antioxidative peptides are low-molecular-weight polypeptides, comprising 2 to 20 amino acid residues, that exhibit various physiological functions (Sarmadi and Ismail, 2010). Xie et. al (2008) have found that the peptides produced from alfalfa leaf proteins exhibited not only high nutritive values but also good antioxidative properties comparable to reduced glutathione, a reference native antioxidant. Food-derived antioxidative peptides have high stability and activity, ease of absorption, and cause no hazardous immunoreaction compared to antioxidant enzymes (Xie et al., 2008). Antioxidative peptides produced from alfalfa leaves can be used as functional foods, nutraceuticals, dietary supplements and constituents of pharmaceuticals.

Achieving a high yield of proteins from alfalfa leaves is an important criteria to meet for economically feasible production of bioactive peptides. Most previous studies in alfalfa protein extraction from fresh leaves involved leaf juice extraction, protein precipitation by heat or acids, followed by protein separation. Final protein yield from alfalfa with respect to the initial protein are typically in the range of 20-60% with varying ratios of green to white proteins (Chiesa and Gnansounou, 2011). Extracting food grade leaf proteins at high yields requires development of a suitable extraction process that targets both soluble and insoluble proteins and is mild enough to preserve amino acids.

The main goal of this proposed study is to investigate 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. To the PI's knowledge, there has been no prior studies of the suitability of cellulase enzymes to improve the protein extractability of alfalfa leaves. In the case of wet distillers grains, a similar approach achieved a protein-rich, fiber

free enhanced DDGS indicating the efficacy of the cellulase enzymes in separating and preserving proteins in DDGS (Kim et al., 2008a, b). Treatment of alfalfa leaves with a proper combination of cell-wall degrading enzymes is expected to increase extractability of proteins by breaking down and exposing the intracellular structures and making plant cells more accessible during the subsequent protein extraction. The schematic diagram of the proposed process is shown in Figure 1.
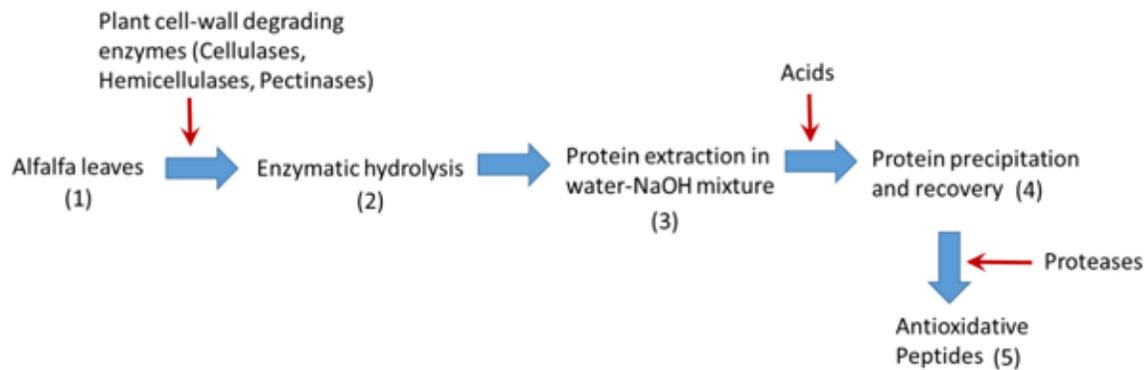
Objectives:
- The objectives of this project are to: 1) Study the effect of cell-wall degrading enzymes on protein extractability of alfalfa leaves and determine the optimal blend of enzymes (cellulases, hemicellulases, pectinases) to maximize protein yields (steps 2 and 3 in Figure 1); 2) Measure the yield and quality (amino acid profile, digestibility) of protein isolates and peptides (steps 4 and 5 in Figure 1) and compare the results to non-enzymatic conventional protein extraction methods; and 3) Provide an experiential learning opportunity for undergraduate agricultural engineering students in bioprocessing of alfalfa for high-value proteins and peptides.