According to the United Nations Food and Agricultural Organization (FAO), global food and feed production needs to increase 25-70% to meet dietary demands of the world's growing population by 2050. This implies animal-based protein demand is also anticipated to increase rapidly. However, sustainable animal protein production is becoming increasingly challenging due to rapidly changing global climate, coupled with burgeoning environmental concerns associated with intensive animal agriculture. Leaf proteins are considered potential alternative protein sources for human and animal consumption. Alfalfa leaves are one of the most important raw leaf protein sources due to the high crude protein content (260 g/kg DM or 2600 kg of protein per ha) and balanced amino acid composition ratio. Producing food-grade leaf proteins requires development of a suitable extraction process resulting in high extraction efficiency, yet mild enough to preserve amino acids.

The study examined use of commercially available, plant cell-wall degrading enzymes (Cellic® CTec2, Viscozyme® L, Pectinex® Ultra SP-L) for alfalfa leaf protein extraction. These were used, individually and in combination, to study effect of plant-cell wall degrading enzymes on alfalfa leaf protein extractability. Extraction yields of alfalfa proteins were compared to those of alkaline extraction without enzymes. Extracted alfalfa proteins were also hydrolyzed into peptides and measured for antioxidative properties.

Results demonstrated the obvious positive effects of certain cell-wall degrading enzyme blends on alfalfa leaf protein extraction. Cellulolytic enzyme preparation (Cellic CTec2) has no effect in improving protein extraction, while both Viscozyme L and Pectinex Ultra SP-L significantly improve protein extractability of alfalfa leaves during the subsequent alkaline treatment. Protein extraction was enhanced by a factor of 1.4-1.6 using Viscozyme L and Pectinex Ultra SP-L at a low enzyme dose (1.5-6 mg/g dry alfalfa) as compared to the protein extraction without enzymes. The recovered protein exhibits a comparable amino acid profile as isolated soy protein, suggesting its potential use as an alternative plant-based protein with its desirable nutritional quality. Enzymatic hydrolysis of alfalfa leaf proteins using a protease (Alcalase) produced peptides with antioxidative activities as measured by the reducing power assay. 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.