

# Opportunities for Alfalfa Protein Extraction

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Jo Heuschele leads the forage biochemistry lab at the USDA-ARS Plant Science Research Unit. Her lab researches plant cell wall characteristics related to animal digestibility. In an effort to further understand the linkage between forage and animal nutrition, she also is looking at leaf protein characteristics and “anti-nutrients” such as saponins. Before joining the Plant Science Research Unit in December 2020, she was a post-doctoral researcher at the University of Minnesota studying oat grain nutrition and lodging resistance. She led a multi-disciplinary team consisting of biologists, civil and dental engineers to determine physical characteristics within an oat stem structure that might increase lodging resistance. During this time, she also mentored both graduate students and high school students in learning about plant research. While at the USDA-ARS Dale Bumpers National Rice Research Center as a post-doctoral researcher, she conducted research on how arsenic differentially enters rice grains. She was part of a science advisory committee that answered to the FDA and USDA in determining acceptable rates of arsenic allowed in the US food supply. She holds a PhD in plant biochemistry and physiology from the University of Minnesota and a Master’s degree in plant ecology from the University of Wisconsin – Eau Claire.

There is a growing demand for protein due to increased population and affluent countries demanding protein rich foods. The majority of plant-based proteins on the market are storage proteins extracted from seeds. These types of proteins are stable prior to extraction and easily extracted with current technologies. However, the most abundant type of plant-based protein resides in plant leaves and stems as the functional protein RuBisCo. When this and other functional proteins are extracted and condensed, they form leaf protein concentrate (LPC). Current methods of LPC extraction include either pulping or juicing the material to release the proteins and then either coagulation, acidification, fermentation, or ultrafiltration to concentrate the soluble proteins. Recovered LPC yields range from 15 to 43% of the original amount of protein found in the plant. These yields are higher than other leafy plants making alfalfa a prime candidate for cultivation for LPC. Alfalfa contains high levels of endogenous proteases which could impact the LPC recovery rates. Proteases breakdown proteins into small subgroups that change protein solubility and the ability to be filtered at a specific size. Our lab is testing how harvest management changes protein size and extraction yields. Three commercial varieties were harvested either immediately dried, immediately juiced, or air dried after cutting. Crude protein extractions were visualized on an acrylamide gel compared with a molecular weight marker standard. The juiced samples had the highest concentration of bands approximately 55 kda in size, supporting that the majority of the proteins within alfalfa leaf tissue is RuBisCo; subunits are approximately 55 kda in size. Immediately dried alfalfa had protein bands at 55kda and smaller with some protein smearing. While aired dried samples showed no protein bands, with extensive protein smearing, suggesting that no intact proteins remain. To further investigate harvest impacts on protein stability we tested seven different harvest including freeze drying and spray drying alfalfa for protein extraction. Our experiments conclude that the harvest method of alfalfa for protein is important for the overall extraction yield.