OPPORTUNITIES FOR EXTRACTION OF PROTEIN FROM ALFALFA

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ABSTRACT

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 in alfalfa 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. Unfortunately, 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 then 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 previous studies that indication most of the proteins within alfalfa leaf tissue are RuBisCo; its 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 little or no proteins remained intact. 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.

Key Words: Leaf protein, RuBisCo, harvest management

POTENTIAL OF LEAF PROTIENS FOR FOOD VS FEED

The increase in the human population and affluent countries demand protein rich foods has caused a renewed interest in alternative protein sources. The focus in plant-based proteins has occurred due to the comparatively lower carbon footprint than animal-based proteins. Most plant-based proteins on the market are from storage proteins extracted from seeds. These types of proteins are stable prior to extraction and are easily extracted with current technologies.

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However, the most abundant type of plant-based protein resides in plant leaves and stems as the functional protein known as RuBisCo. When this and other functional proteins are extracted and condensed, they form leaf protein concentrate (LPC).

Utilizing LPC as a protein source is not new. During World War II there was a push to extract leaf protein to address food shortages. Money and time were invested into research to extract usable protein from leaves of various crops. A pilot refinery using the Pro-Xan II method of extraction was even built in California to extract and process alfalfa protein in the 1970's. Today many countries have pilot, demo, and industrial scale biorefineries that process leaf tissue to extract proteins that are utilized for the animal feed industry (Fiorentini & Galoppini, 1981).

However, LPC has not made it into the mainstream plant-based protein food markets for various reasons. Originally the plant-protein market was small and technologies for extracting proteins from seeds like soy surpassed the leaf extraction technology. The gap was further widened due to the cost benefit ratio of the inputs and low LPC extraction yields compared to seeds. 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 species of plant. The remaining insoluble proteins can be recovered and utilized in the feed market.

Alfalfa has the highest yields than other leafy plants ranging from 20 – 43% making it a prime candidate for LPC cultivation. The amino acid profile of alfalfa is similar to soy and meet the FOA requirements for a complete protein. Additionally, the functionality characteristics of alfalfa LPC is similar to egg whites with no adverse flavors (Knuckles & Koler, 1982). Unfortunately, there are numerous challenges that need to be addressed before alfalfa LPC can become a mainstream protein source.

A MAJOR CHALLENGE TO ALFALFA PROTEIN

The one of the major challenges with marketing alfalfa LPC is the variability in protein yield. 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. They proteases are active across a wide range of pH's suggesting there are pH specific classes of proteases within alfalfa (Scalet et al. 1984). Our lab began investigating protein extraction, by comparing juiced alfalfa with hayed alfalfa. While the crude protein levels measured by NIR were the same, crude protein extractions visualized on an acrylamide gel compared with a molecular weight marker were not. Field dried hay samples showed no protein bands, but had smearing, suggesting that no intact proteins remain. Juiced alfalfa had protein bands at 55kda and smaller with some smearing at less than 3 kda. RuBisCo subunits are approximately 55 kda. We additionally tested immediately dried alfalfa at 140°F for three days. Those samples also showed clear bands at 55 kda. To determine if there was a possible genetic component to the degradation of the proteins, we tested three modern cultivars. All cultivars responded the same to post harvest treatments of juicing, air and field drying. The break down in protein during harvest is not a problem for ruminants as they can still utilize the

amino acids to create microbial proteins. Monogastrics, however, require some intact proteins for digestion to maintain nitrogen use efficiency and balance within the gut (Eugenio et al, 2022).

Protein yields were still low and variable when the material was moved to the concentration step. All commonly used methods of concentrating soluble proteins require the proteins to be incubated in water sometime during the process (Hadidi et al 2019). Dried crude protein alfalfa samples that showed strong bands previously were exhibiting degraded protein smears after concentration steps. We hypothesized that reconstituting alfalfa in water reactivated the proteases at both high and low pH. We found the longer the sample was incubated in aqueous solution the more protein was degraded irrespective of pH. Inhibiting degradation during concentration is important to maintaining protein yield.

Finding ways to maintain alfalfa LCP structure and size before emulsification is also a challenge. While heating of the sample aggregated and stabilized the protein, it did not improve the solubility of the protein and therefore, preventing the protein from being used in any clear liquid final product. Stability and consistency of the protein needs to be investigated along with the development of end products. Solubility of the protein might not matter if the end product requires a curd instead of the solubilized form of protein.

Our lab continues to evaluate how harvest management changes protein size and extraction yields with additional types of methods to prevent protease degradation during protein concentration.

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