Wet Fractionation for Improved Utilization of Alfalfa Leaves

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ABSTRACT. *Utilization of alfalfa could be greatly improved if the protein-rich leaves were efficiently separated and preserved from the fibrous stems. This work envisions a new harvest scheme combining three processes: mechanical leaf separation, pressing, and anaerobic storage. To quantify the effectiveness of leaf dewatering, experiments were conducted in which leaves were pressed in a replicated factorial design, including maceration and four levels of backpressure. The amount of press filtrate extracted varied proportionally with press backpressure from 211 to 612 L Mg^{-1} fresh leaves and was composed of about 90% water. The resulting partially dewatered leaves were successfully ensiled and were found to be chemically similar to high-quality, whole-plant alfalfa silages. Additionally, we demonstrated that nutritionally valuable components in the press filtrate could be conserved by anaerobic storage. Based on our work, protein and lactic acid could be obtained from the ensiled press filtrate in quantities as high as 300 and 143 kg ha^{-1}, assuming an average annual leaf yield of 10 Mg ha^{-1} and optimal process conditions. However, more work is necessary to determine these values.*

Keywords. Alfalfa, Fermentation, Fractionation, Lucerne, Protein, Silage, Wet fractionation.

Forage is the main ingredient in the diet of U.S. dairy cows. Over 15.1 million ha of dry hay and haylage and 2.0 million ha of corn silage meet the dietary needs of the 9.1 million cows in the U.S. (USDA-ERS, 2011). The U.S. dairy industry is the second largest livestock sector, behind meat production, and has a farm value equal to corn. The annual dairy industry output is USD $48 billion, supporting more than 300,000 jobs (USDA-NASS, 2008). The raw material and the subsequent global competitiveness of this economic engine are dependent on high-quality forage.

Alfalfa-corn rotation strikes a balance of the protein and energy needed for maximum animal productivity while minimizing crop inputs and the energy footprint of the dairy. For example, alfalfa supplies its own nitrogen (N) fertilizer and can provide about 75% of the N needed during the following two years of corn planting. Because the need for synthetic fertilizers is reduced, there is less risk of contamination of the groundwater and surface water, and less greenhouse gas emissions. In fact, alfalfa, because of its deep root

system, is a great crop for sequestration of carbon to the soil. Additionally, strip-cropping practices enable alfalfa stands to maintain spring ground cover between fall-harvested corn strips, especially in areas that may be more prone to soil erosion.

Recently, alfalfa harvest management has focused on early cutting for quality to enhance feed intake of high-producing cows (Rankin, 2011). However, this practice not only limits inclusion in dairy rations, because of the environmental risks associated with overfeeding protein, but also limits production of fiber, a valuable component of the ruminant diet. Furthermore, harvesting immature alfalfa (early bud stage) narrows the harvest window. As a result, producers are forced to take on greater weather risk and subsequent crop loss due to rain. Harvesting at earlier maturities also results in more frequent harvests. For example, Wisconsin alfalfa producers who once debated taking a fourth cutting now consider a fifth cutting. Frequent cuttings increase the harvest-related costs, and the resulting equipment traffic reduces alfalfa stand life. The result of this management practice is a lower yielding, more costly, and compositionally variable feedstuff that has limited ration inclusion rates.

If alfalfa leaves and stems could be harvested and stored separately, both fiber and protein yield could be optimized (Talbott et al., 2005) because alfalfa leaves change little in quality over the development of the plant, whereas stems continually decrease in quality (Buxton and O'Kiely, 2003). The producer could then combine the two fractions in proportions that optimize the dietary requirements of the target animal. For example, lactating dairy cows would receive proportionately higher levels of leaves, whereas non-lactating heifers would receive more stems. This utilization scheme would enable higher ration inclusion rates and optimal use of alfalfa protein and fiber, providing a balanced and cost-effective crop production scheme for the dairy producer.

Long-term stability of anaerobically stored silage depends on fermentation dominated by lactic acid bacteria (LAB). When alfalfa is harvested with a moisture content that is sufficiently low, fermentation of carbohydrates by LAB is favored over clostridia. However, direct-harvested leaves have high moisture content; therefore, an alternative approach has been employed, exploiting the fact that LAB can tolerate lower pH levels than clostridia (Shinners et al., 2007; Muck et al., 2010). Muck et al. (2010) and Shinners et al. (2007) reported experiments in which alfalfa leaves were successfully preserved by lowering the initial pH using formic acid. However, at dry matter contents less than 230 g kg^{-1}, leaf fermentation was dominated by clostridia, which produced unstable and unpalatable silage (McDonald, 1981a; Muck et al., 2010). This is partly because the buffering capacity of alfalfa leaves is different from that of the whole plant, limiting the ability of formic acid to create a pH favorable to LAB (Muck et al., 2010). This problem could be remedied by increasing the treatment level, but the result may quickly become cost-prohibitive due to chemical cost. Additionally, effluent containing valuable plant nutrients would be lost from the wet alfalfa leaves (Rotz et al., 1993).

We propose an alternative strategy for producing leaf silage. Reducing leaf water content could maintain the benefits of direct harvesting without the need for pH adjustment and effluent loss. Additionally, if only the leaves are dewatered, then only half of the total plant mass needs to be direct-harvested, transported, and dewatered. Dewatering only half of the plant mass effectively doubles the capacity of the press per unit area, thereby reducing capital, energy, and press filtrate handling and storage costs. However, past experience indicates that the primary mechanism for mechanical dewatering is cell rupture, so selectivity for water may not be possible, as both soluble and suspended proteins, fats, and carbohydrates will be fractionated (Jorgensen and

Koegel, 1988). Therefore, utilization of the press filtrate stream and its components may be necessary for the overall success of the system.

If selectivity for water cannot be obtained, then the press filtrate will need to be utilized on or off the farm. Past work has employed pressing to fractionate protein and other biochemicals from plant fiber (for reviews, see Telek and Graham, 1983; Jorgensen and Koegel, 1988; Kromus et al., 2006). This approach could be beneficial on the farm if greater value could be realized for the separated fractions. Sugars, lipids, vitamins, and drug ingredients have been successfully fractionated, but most work in alfalfa has focused on soluble protein.

Based on previous work, it is our hypothesis that the process variables of a rotary extrusion macerator and single-screw press can be managed to: (1) selectively remove water and thus minimize the loss of nutritive value of press cake silages, and (2) increase the amount of protein removed to produce value-added co-products. To this end, we conducted experiments in which leaves were pressed in a replicated factorial experiment that included maceration and four levels of discharge cone backpressure on a single-screw press. Furthermore, we investigated fermentation of the press filtrate for recovery of nutrients lost in the dewatering process for on- or off-farm utilization. Press cake and filtrate fractions were stored separately and anaerobically in individual laboratory silos.

Materials and Methods

The factorial experiment was conducted on 13 July and 18 August 2011 in second and third cuttings of a three-year-old, 4 ha stand of alfalfa (43° 18′ 6.75″ N, 89° 21′ 12.78″ W) at the University of Wisconsin Arlington Agricultural Research Station located in Arlington, Wisconsin. In each case, alfalfa was in the early flower stage of maturity. Leaves were harvested using the methodology and machine configuration described by Shinners et al. (2007). A picking-reel tine to ground speed ratio of 13:1 was maintained during harvest. Tines were set to engage 75% of the plant height in an effort to minimize leaf contamination with stems. Whole-plant and leaf yields were estimated at four 0.25 m^2 locations for each cutting. Pre- and post-harvest leaf-to-stem ratios were determined by hand separation of stems from leaves, petioles, buds, and flowers (Albrecht et al., 1987). Harvested leaves were collected in 120 L containers and transported to Madison, Wisconsin, for processing. Prior to processing, the leaves were spread out on a tarp and treated with a lactic acid bacterial (LAB) inoculant (Ecosyl MTD/1, Ecosyl Products Ltd., Stokesley, U.K.) at a rate of 10^5 colony-forming units (CFU) g^{-1} alfalfa, according to the manufacturer's specification.

The leaves were dehydrated with a 15.24 cm varying-pitch interrupted single-screw press (S-Press model RSP-6-H2, Rietz Mfg. Co., West Chester, Pa.) operated at 19 rpm. A stainless steel screen partially fractionated solids from the expressed press filtrate (508 µm, 23% open area). The outlet of the press could be variably restricted by a perforated stainless steel cone actuated by a 79 mm diameter pneumatic cylinder. Maceration was completed prior to pressing with an experimental rotary extrusion (die ring) macerator (Nelson, 1980). The macerator was operated with an orifice size of 12.7 mm, open area of 38%, die ring diameter of 410 mm, roller diameter of 257 mm, and surface speed of 5.0 m s^{-1}. Macerator performance was monitored by conductivity index (Kraus et al., 1999).

Upon completion of pressing, both press cake and filtrate were stored anaerobically. Press cake samples were densified by hand into "mini-silos" (1 L tulip jars, J. Weck

GmbH and Co., Oflingen, Germany), consistent with the method of Pflaum et al. (1996). Storage conditions were anaerobic, 22°C, and 200 kg DM m^{-3} density. For each run, 45 mL of fresh press filtrate was collected in 50 mL conical centrifuge tubes and frozen at -20°C for later analysis. A 200 mL aliquot of press filtrate from each run was also stored anaerobically in a borosilicate glass canning jar with a rubber seal (0.25 L cylindrical jar, J. Weck GmbH and Co., Oflingen, Germany). All mini-silos were weighed to monitor dry matter loss.

After 7 days, the press filtrate mini-silos were homogenized by hand-shaking and transferred to 250 mL centrifuge bottles. At this point, a 15 mL aliquot was taken and frozen. The remaining sample was centrifuged at $10,400 \times g$ for 10 min to separate suspended solids. The resulting pellet and supernatant fractions were frozen in separate 250 mL bottles. The pellet was then freeze-dried, broken up with a spatula, and stored in a sealed container for subsequent analysis.

All compositional data in this article are expressed on a dry matter basis. Dry matter was determined as loss on drying in a forced-air oven at 55°C for 48 h. Ash content was determined as residue remaining after combustion at 500°C for 4 h. The solids of the liquid fractions were determined by ASTM Method E1756-08 (ASTM, 2008).

At completion of a 90-day anaerobic storage period, the press cake mini-silos were weighed, emptied, and mixed by hand in a plastic tub. Three subsamples were taken. The first, a 20 g subsample was suspended in 200 mL of distilled water and homogenized using a laboratory grinder (model B-400, Büchi Labortechnik AG, Flawil, Switzerland). A 1 mL aliquot was taken, frozen at -20°C, and used to measure fermentation products following the method described by Muck and Dickerson (1988). Fermentation acids (succinic, lactic, acetic, propionic, and butyric) were determined by high-performance liquid chromatography (HPLC). The HPLC system was configured with an analytical column (Supelcogel C-610H, Sigma-Aldrich, St. Louis, Mo.), a guard column, and a UV/Vis detector set at 205 nm. The eluent was 0.1% H_3PO_4 solution at 0.6 mL min^{-1} and 50°C. A second subsample of 40 g was frozen at -20°C and subsequently freeze-dried. Dried samples were ground in a vortex mill (Udy Corp., Fort Collins, Colo.) through a 1 mm screen. These subsamples were used for all subsequent analyses. The remainder of the mini-silos' contents comprised the third subsample, which was frozen at -20°C in plastic bags.

Press cake ethanol concentrations were determined by centrifuging a water extract to remove suspended solids. The sample was diluted with reverse osmosis (RO) water, filtered through a 0.2 μm nylon syringe filter, and analyzed on an ion chromatography system (Dionex ICS-3000, Thermo Scientific, Sunnyvale, Cal.). The system was equipped with an IonPac ICE-AS1 analytical column (4×250 mm) and an integrated amperometric detector with disposable gold electrode. The eluent was 100 mM methanesulfonic acid at 0.25 mL min^{-1}, and the column compartment was kept at 30°C.

Crude cell wall protein content was analyzed using a macroelemental combustion analyzer (VarioMax CN, Elementar Americas, Mt. Laurel, N.J.) to determine sample nitrogen content. A cell wall sample of approximately 50 mg was used for analysis. Glutamic acid was used as a reference standard. Crude protein was calculated as the percent nitrogen multiplied by a factor of 6.25, according to the manufacturer's protocols. Crude fat was determined by diethyl ether extract and was automated utilizing a solvent recovery system (Soxtec 2047, Foss A/S, Hillerod, Denmark) (AOAC, 2000).

Crude fiber was determined by a modified version of the Uppsala total dietary fiber

method (Theander et al., 1995). Samples of fresh leaves for each silo were weighed into 50 mL conical centrifuge tubes (25 mg). Tris buffer (50 mM, pH 6.7) was added, and each sample was placed in a 90°C water bath for 2 h to gelatinize the starch. The samples were transferred to a 55°C water bath and incubated for 2 h after adding amylase (Sigma A3403, 10 U tube^{-1}) and amyloglucosidase (Fluka 10115, 10 U tube^{-1}) for starch removal. After incubation, a subsample of the liquid fraction (4 mL) was taken for starch determination measured as glucose. Ethanol (EtOH, 95%) was added to each tube to produce a final EtOH concentration of 80%. Samples were stirred with a spatula before centrifuging at $3200 \times g$ for 15 min. Insoluble residues and pellets recovered from the starch extraction procedure were washed extensively (1 mL solvent g^{-1} fresh tissue) with a solvent series consisting of 80% EtOH (4×) and acetone (2×) to remove cytoplasmic contaminants (Hatfield, 1992; Hatfield et al., 1999). The retained insoluble residues, mainly cell walls, were allowed to air-dry under a hood and then dried overnight in a 55°C oven prior to weighing. The quotient of this weight, corrected for crude protein compared to fresh dry weight, is reported here as crude fiber.

Starch content was determined after the gelatinization and removal step of the previously described crude fiber analysis and before an alcohol washing of the 4 mL aliquot was taken to measure starch. Hydrolyzed starch was then measured as free glucose colorimetrically (505 nm) using a glucose-oxidase-peroxidase-chromogen system (Karkalas, 1985). A standard curve was developed using glucose in a range of 10 to 200 µg mL^{-1}. Total soluble sugars present in the sample prior to starch hydrolysis were determined by the phenol-H_2SO_4 method of Dubois et al. (1956). Briefly, 250 µL of sample was mixed with 250 µL of 5% phenol solution followed by 1.25 mL of 18 M H_2SO_4. Samples were mixed thoroughly using a vortex mixer and allowed to cool (20 min) before reading at 490 nm. Again, a standard curve was developed using glucose in a range of 10 to 200 µg mL^{-1}.

Total saccharides determination was accomplished by hydrolyzing a sample from the liquid fraction with a 4 wt% sulfuric acid solution at 121°C for 1 h (TAPPI, 2009; Sluiter et al., 2011). The hydrolyzed sample was neutralized with 1.2 N NaOH, diluted with RO water, filtered through a 0.2 µm nylon syringe filter, and analyzed with an ion chromatography system (Dionex ICS-3000, Thermo Scientific, Sunnyvale, Cal.). The system was equipped with a carbohydrate column (CarboPac PA20, Thermo Scientific) and an integrated amperometric detector with disposable gold electrode. The eluent and post-column eluent used were RO water at 0.35 mL min^{-1} and 0.5 mol L^{-1} NaOH, respectively. The column compartment was kept at 30°C.

Experimental variables consisted of four cone backpressures (69, 138, 276, and 552 kPa) and two levels of maceration (macerated and unmacerated). Measured responses included press filtrate yield, protein concentration and recovery, and press cake fermentation profile. The experiment was replicated in four completely randomized blocks in each of the two cuttings. Significance of experimental factors on measured responses and confidence intervals were evaluated using R (version 2.14.0, The R Foundation for Statistical Computing, Vienna, Austria). The results were analyzed by the *aov* procedure. Differences between treatment means were tested using Student's t-test. Statistical significance was recognized for $p < 0.05$. Press filtrate, water, and protein yields were modeled using the *lm* procedure; linear, quadratic, and logarithmic models were considered. The *nls* procedure was used for data that were better described by non-linear models.

Table 1. Composition of harvested alfalfa leaves for second and third cuttings.[a]

Cutting	Dry Matter (g kg^{-1})	Ash (g kg^{-1} DM)	Crude Fiber (g kg^{-1} DM)	Free Sugar (g kg^{-1} DM)	Starch (g kg^{-1} DM)	Crude Protein (g kg^{-1} DM)	Fat (g kg^{-1} DM)
Second	192 ±3	98 ±3	328 ±10	53 ±8	25 ±6	248 ±5	36 ±4
Third	246 ±2	87 ±5	285 ±16	61 ±10	84 ±17	226 ±7	29 ±4

[a] Values are means ±95% confidence intervals.

Results and Discussion

Second and third cutting whole-plant alfalfa yields were 2.6 and 2.4 ±0.3 Mg ha^{-1}, respectively. Yields agreed well with the values of 2.7 and 2.3 Mg ha^{-1} reported for second and third cuttings by the Wisconsin Alfalfa Yield and Persistence trial (Rankin, 2011). Leaf-to-stem mass ratio (LSR) averaged across cuttings was 2.3 ±0.5, which was much higher than any of the LSRs reported by Albrecht et al. (1987) but similar to more recent data presented by Duncan (2010). This could indicate that our crop was less mature than the -1 week bud stage, or it could be the result of cultivar differences. The leaf harvester produced a leaf-to-stem ratio of 19 ±1, which was higher than the ratio of 9 reported by Shinners et al. (2007); however, our emphasis was on a pure leaf fraction rather than maximizing harvested mass. Although both cuttings were harvested at the early bud stage of maturity and at a mid-morning time, harvested leaf moisture varied significantly between the two cuttings. Harvested leaf moisture content was 808 and 752 ±23 g kg^{-1} in the second and third cuttings, respectively (table 1). However, this range is less than the 766 to 832 g kg^{-1} observed by Muck et al. (2010) over three leaf harvests from June to August.

Dewatering Characteristics of Leaves

Press filtrate yield increased proportionally to the press backpressure and was not correlated with maceration, pretreatment, or cutting. In each cutting, the pressing characteristics of leaves followed this supposition, irrespective of initial leaf moisture (fig. 1). Leaf moisture was reduced from 808 to 690 g kg^{-1} and from 752 to 631 g kg^{-1} for the second and third cuttings, respectively. Therefore, total press filtrate yield varied from 211 to 612 L Mg^{-1}. These results agree well with preliminary work conducted by K. J. Shinners (unpublished data, 2011, Department of Biological Systems Engineering, University of Wisconsin, Madison, Wisc.). Shinners conducted pressing tests on alfalfa leaves with the same single-screw press used here on four separate days over two cuttings. Average initial leaf moisture was 811 g kg^{-1}, and final moisture obtained was 675 g kg^{-1}. However, these results are slightly lower than the reduction from 740 to 580 g kg^{-1} reported by Knuckles et al. (1972) for whole-plant alfalfa, although the experimental setup of the single-screw press was not detailed. Concurrent to backpressure, the impact of leaf maceration was studied. Maceration with a rotary extrusion macerator yielded a conductivity index of 0.56 ±0.03. Maceration had little effect on press filtrate removed, with no detectable trends across various press backpressures (fig. 1). This result is also in agreement with Shinners' work, which used a hammer mill that likely obtained a higher level of cell rupture, although conductivity index was not reported.

Because cutting and level of maceration did not significantly affect press filtrate yield, a nonlinear model was developed on backpressure alone to describe the fractionation characteristics of alfalfa leaves over the range of pressures tested:
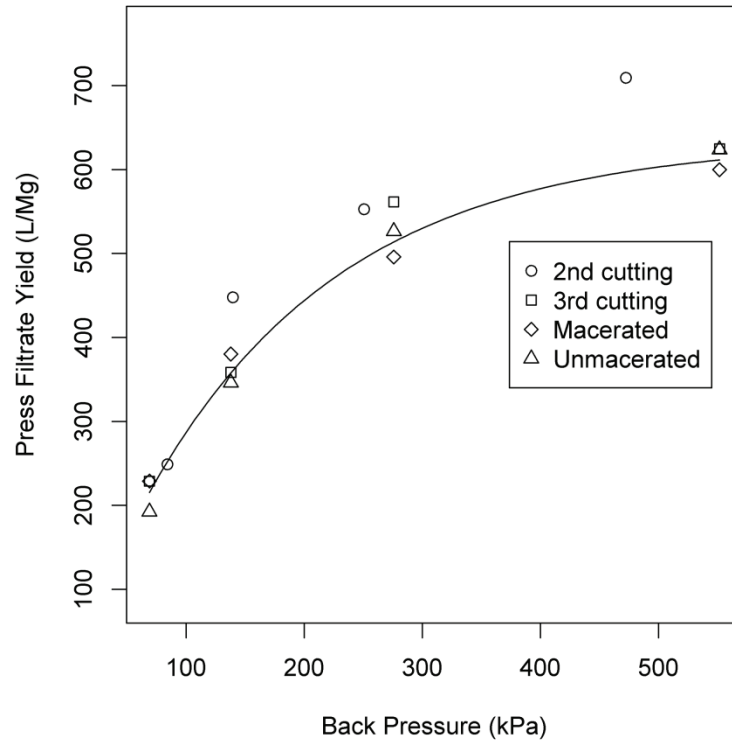
Figure 1. Press filtrate yields for two cuttings and levels of maceration with varying levels of press backpressures. Data points represent means of individual treatment combinations. The regression line represents predicted values based on computations from equation 1, where the residual standard error is 84.0. Press filtrate exhibited a linear relationship with water, with a slope of 0.899 and RSE of 3.23.

$$y(p) = A(1 - e^{-Bp}) \tag{1}$$

where y represents press filtrate yield (L Mg^{-1}) of fresh material, which depends on cone backpressure (p, kPa), and coefficients A and B were determined to be 634 ±6.52 and 0.00602d ±0.000155, respectively. In both cases, coefficients A and B were significant at p = 0.001. Linear and intercept terms introduced into the model only slightly improved regression performance, as measured by R^2 and residual standard error (RSE). Consequently, these terms were omitted to prevent overfitting. The model described the range of observed pressures with R^2 of 0.77 and RSE of 84.0.

Press filtrate yield was related to dewatering by accounting for the solids in the press filtrate. Based on this analysis, press filtrate yield could be related to water extracted by the linear model. The slope was estimated to be 0.899 ±0.000194. This model described dewatering over the range of pressures with R^2 of 1.00 and RSE of 3.22. The fitted model performance and the reliance on a single dependent variable demonstrated the inability to alter the specificity for water for the variables studied.

Press Cake Silages

Fermentation profiles indicated that leaf press cake silages were well preserved by anaerobic fermentation (table 2). Organic acid accumulation reduced press cake pre-

Table 2. Organic acid, pH, and ethanol contents of second and third cuttings of wet fractionated alfalfa leaves (press cake) after varied levels of pressing and anaerobic storage (ensiling) for 90 days.[a]

Cutting		Press Filtrate Yield (L Mg[-1])	Dry Matter (g kg^{-1})	pH	Organic Acids and Ethanol (g kg^{-1} DM)			
					Succinate	Acetate	Lactate	Ethanol
Second		216 a	213 a	4.60 a	3 a	21 a	71 a	33 a
		411 b	250 ab	4.56 a	3 a	17 a	54 ab	25 a
		513 b	267 b	4.56 a	3 a	16 a	53 ab	27 a
		665 c	290 b	4.51 a	2 a	14 a	49 b	25 a
	LSD	122	46	0.19	2	7	21	15
Third		255 a	276 a	4.53 a	4 a	17 a	61 a	28 a
		400 a	301 ab	4.46 a	3 a	16 a	52 a	26 ab
		625 b	355 bc	4.46 a	3 a	12 a	45 a	23 bc
		694 b	364 c	4.43 a	3 a	11 a	44 a	20 c
	LSD	186	59	0.46	2	9	30	4

[a] Within a column, values followed by different letters are significantly different at $\alpha = 0.05$.

ensiling pH from 6.30 ±0.02 to 4.51 ±0.06. The pH was not found to vary significantly by treatment. Butyric acid concentrations were below the detection limit (1 g kg^{-1} DM), indicating low activity of clostridia, which agrees with the average dry matter loss observed at 10 ±0.3 g kg^{-1} DM. According to R. Muck (personal communication, 2009, USDA-ARS U.S. Dairy Forage Research Center, Madison, Wisc.), the dry matter loss reported here should indicate a well-sealed lab silo rather than what could be expected at the field scale.

Individual organic acids indicate a numerical trend of lower concentrations as more press filtrate was removed from the leaf material. However, variability between replicate lab silos limited our ability to detect significant changes. Comparing our dry matter concentration of 213 g kg^{-1} press cake silage to the 228 and 229 g kg^{-1} leaf silages reported by Muck et al. (2010), our final pH was slightly higher than the 4.32 and 4.50 pH levels that they obtained. In addition, lactic acid was between 62.4 and 78.9 g kg^{-1} DM, and acetic acid was between 18.8 and 26.1 g kg^{-1} DM, as observed in the earlier study. However, ethanol was significantly higher than observed by Muck et al. (2010). Both our data and those of Muck et al. (2010) are contrary to the fermentation regimes observed by Russell et al. (1978) in whole-plant alfalfa press cake silages. The success of leaf-only fermentation is likely the result of the relatively higher concentration of fermentable carbohydrates compared to whole-plant press cakes.

In agreement with the fermentation profile data, alfalfa leaf press cake composition was well-preserved by the ensiling process (table 3). As a result, silage quality compared

Table 3. Composition of wet fractionated alfalfa leaves (press cake) obtained at cone backpressure of 552 kPa and without maceration for second and third cuttings before and after 90 days of storage.[a]

Cutting	Dry Matter (g kg^{-1})	Ash (g kg^{-1} DM)	Crude Fiber (g kg^{-1} DM)	Free Sugar[b] (g kg^{-1} DM)	Starch (g kg^{-1} DM)	Crude Protein (g kg^{-1} DM)	Fat[b] (g kg^{-1} DM)
Second							
Pre-ensiled	308 a	73 a	442 a	28 ±4	38 a	222 a	-
Ensiled	290 a	79 a	411 b	-	19 b	229 a	52 ±2
LSD	43	8	16	-	4	12	-
Third							
Pre-ensiled	376 a	67 a	316 a	37 ±6	107 a	229 a	-
Ensiled	364 a	68 a	325 a	-	80 b	220 a	63 ±6
LSD	34	6	23	-	11	9	-

[a] Within a column, values followed by different letters are significantly different at $\alpha = 0.05$.
[b] Values are means ±95% confidence intervals. Dash (-) denotes that constituent was not measured.

favorably to conventional whole-plant alfalfa silages when considering crude protein, starch, ash, and fat concentrations. Average composition for legume haylage reported at a regional forage testing laboratory was 425, 205, and 32 g kg^{-1} DM for crude fiber (NDF), crude protein, and fat, respectively (DLFS, 2011). However, leaf press cake silages differed from whole-plant alfalfa silages in ash and starch contents. Starch was 30% higher than the regional average and exceeded the highest concentration of 24 g kg^{-1} DM reported by DLFS (2011). Press cake ash was 39% lower than the regional average but was within the range observed. We also observed that the amount of ash was lower than that of fresh leaves (table 3). Based on this observation and past effluent work reported by McDonald (1981b), we speculate that some inorganic elements were solubilized and removed by the pressing process.

Protein Extraction

As backpressure was increased, more press filtrate was removed from the alfalfa leaves while the concentration of protein remained unchanged (p = 0.0589), and thus the protein extraction level increased (p < 0.001). These data also indicate that protein concentration (p = 0.002) and recovery (p = 0.0458) could be improved by maceration (fig. 2). Therefore, models for each level of maceration were developed that expressed protein extracted per kg of fresh leaf protein as a function of press filtrate yield, from 216 to 694 L Mg^{-1}. The model's slope and intercept were estimated to be 0.265 ±0.04 and 73.1 ±0.04, respectively, for unmacerated leaves and 0.331 ±0.04 and 73.1 ±0.04, respectively, for macerated leaves. The linear models fit the data with R^2 of 0.96 and 0.99 and RSE of 12 and 7.0 for unmacerated and macerated leaves, respectively.

In summary, a maximum of 30% of the total leaf protein was recovered from the
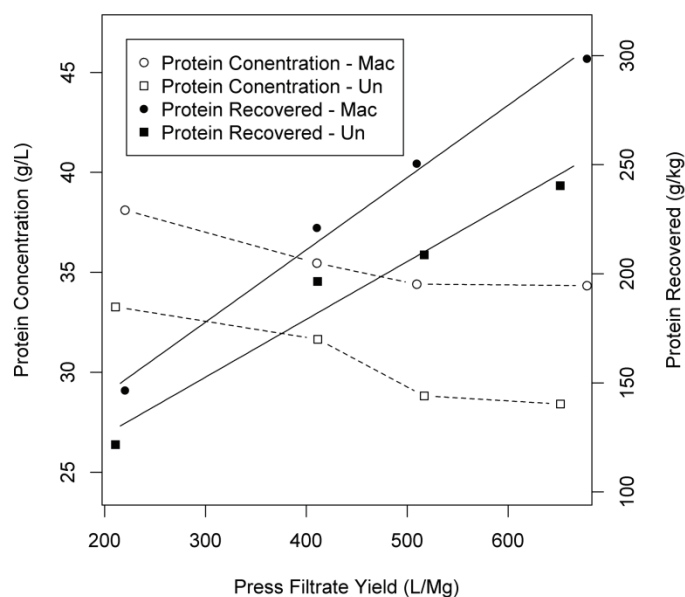
Figure 2. Press protein concentration and recovery versus yield for macerated (Mac) and unmacerated (Un) alfalfa leaves averaged over second and third cuttings. Solid lines depict fitted models for protein recovered from macerated (Mac) and unmacerated (Un) alfalfa leaves. The RSE for macerated and unmacerated samples is 12 and 7.0, respectively.

leaves after pressing. This result agrees well with whole-plant alfalfa protein extraction yields reported in the literature. Edwards et al. (1977) were able to extract 23% of the total protein with a twin-screw press, and a 38% yield was achieved by Marcotte et al. (2002) after maceration and pressing through a single-screw press. Based on past and current data, one can expect similar protein fractionation characteristics for alfalfa leaves and whole-plant alfalfa.

Unfortunately none of the press levels studied improved selectivity for water. Therefore, although fermentation of leaf press cakes was successful at low levels of dewatering, more than 10% of the leaf protein may need to be recovered from the press filtrate, stored, and fed or marketed in order for the system to be an economical alternative to field wilting.

Press Filtrate Preservation and Fractionation

Naturally occurring monomeric and polymeric sources of pentoses and hexoses in the press filtrate can be fermented to lactic acid (Stahmann, 1976). Statistical differences in pentoses (p = 0.010) and hexoses (p = 0.006) were detected for maceration in our study. However, actual concentrations of pentoses and hexoses only varied from 1.8 to 2.0 g L^{-1} and from 23 to 25 g L^{-1}, respectively, with higher concentrations of sugars observed in press filtrate liberated from macerated leaves. Seven days of anaerobic storage resulted in fermentation of 76% ±5.8% of hexoses and pentoses to lactic acid (fig. 3). Lactic acid concentrations ranged from 12 to 21 g L^{-1}, but the differences were not well correlated with the press and maceration pretreatment variables studied. These yields were higher than the 12 g L^{-1} average but within the range of 7 to 20 g L^{-1} reported by Ajibola (1984). The average lactic acid concentration for maximum press filtrate yield and maceration
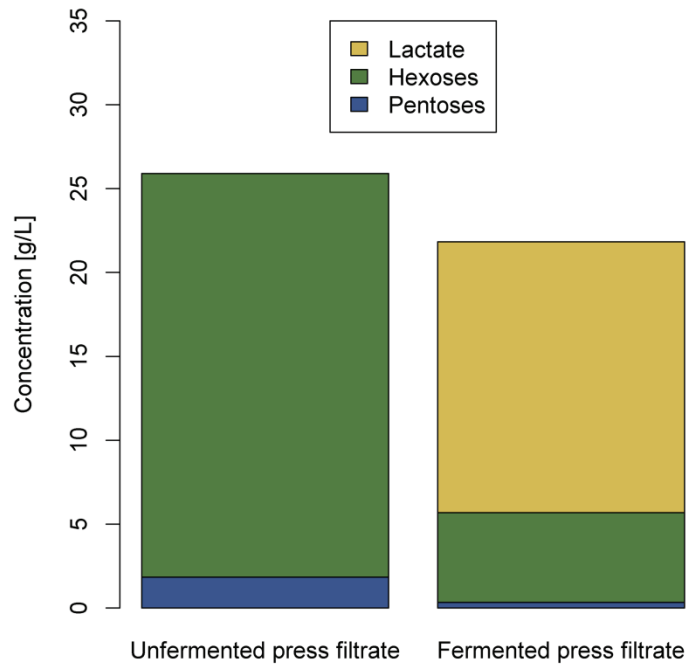
Figure 3. Average composition of press filtrate realized after maceration and pressing through a single-screw press with cone backpressure of 552 kPa before and after 7 days of anaerobic storage.

Table 4. Composition of press filtrate obtained at backpressure of 552 kPa and without maceration before (filtrate) and suspended solids after (fermented) 7 days of storage for second and third cuttings.[a]

Cutting	Solids (g L^{-1})	Ash (g kg^{-1} DM)	Crude Fiber (g kg^{-1} DM)	Free Sugar[b] (g kg^{-1} DM)	Starch (g kg^{-1} DM)	Crude Protein (g kg^{-1} DM)	Fat[b] (g kg^{-1} DM)
Second							
Filtrate	96	159 a	54 a	174 ±33	61 a	272 a	-
Fermented	-	61 b	120 b	-	59 a	340 b	162 ±21
LSD	-	17	8	-	6	30	-
Third							
Filtrate	83	153 a	42 a	228 ±26	80 a	216 a	-
Fermented	-	76 b	116 b	-	147 b	289 b	126 ±17
LSD	-	30	14	-	11	18	-

[a] Within a column, values followed by different letters are significantly different at $\alpha = 0.05$.

[b] Values are means ±95% confidence intervals. Dash (-) denotes that constituent was not measured.

pretreatment conditions was 16 ±2.0 g L^{-1}.

Solids obtained from press filtrate fermentation are compositionally valuable but have limited yield potential, given the range of process variables studied. The pre-ensiled press filtrate composition obtained in our study (table 4) had lower concentrations of protein but similar concentrations of ash, fiber, and fat compared to whole-plant press filtrate, as summarized by Jorgensen and Koegel (1988). Fermentation of the press filtrate converted sugars to lactic acid, as previously mentioned, thereby precipitating the protein and solubilizing a portion of the ash fraction. As a result, the ensiled solids contained concentrated fiber, protein, and fat.

Based on these data, lactic acid could add value to the current alfalfa production system. This determination was made by assuming average yield conditions, i.e., an annual yield of 10 Mg wet leaves ha^{-1}, press filtrate yield of 235 to 680 L Mg^{-1}, and a fermentation yield of 12 to 21 g lactic acid L^{-1}. The result is 28 to 143 kg lactate ha^{-1}, for which we estimated the maximum market value at USD $34 to $175 ha^{-1} year^{-1}. For context, a recent study reported alfalfa-corn bioenergy rotation profitability to range from significant losses to USD $650 ha^{-1} year^{-1}, depending on input costs and market values (Vadas et al., 2008). Furthermore, we estimate that lactic acid production could be increased by 13 to 88 kg ha^{-1} year^{-1} by hydrolysis and fermentation of the starch observed in the ensiled press filtrate, increasing the value to USD $50 to 321 ha^{-1} year^{-1}. However, this value would only be realized after purification to food grade. Danner et al. (2000) showed that obtaining food-grade lactic acid from silages is problematic, even with modern purification techniques. However, more recent work combining biotechnical and chemical processes could lead to lower production cost of purified lactic acid (Kamm et al., 2009). Furthermore, it may be necessary, depending on end use, to select lactic acid bacteria for optimum isomer ratio. Our current understanding leads us to believe that lactic acid obtained from this process would be best utilized as an animal feed after protein solids have been removed.

Summary

Field fractionation and dewatering of alfalfa leaves has distinct advantages over past methods that focused on the whole plant. First, since leaves account for, at most, half of the total plant mass, the capacity of the press is doubled per hectare, reducing capital costs needed to meet today's harvest rates.

Second, this process led to successful ensiling of alfalfa leaves as partially dewatered press cake, thereby avoiding the need for field wilting. Press cake silages were chemically similar to high-quality, conventional alfalfa silages. This contrasts with whole-plant press cake silages, in which elevated concentrations of butyric acid were observed. We attribute this primarily to the abundance of free sugars and starch, naturally present in leaves, and that these carbohydrates were only partially removed from the press cake. However, further work is necessary to determine the economics of dewatering leaves relative to alternatives such as acidification or field wilting.

Third, field separation of stems and leaves followed by pressing and fermentation could be used to concentrate protein relative to fiber and would have utility in creating protein-rich product streams from alfalfa biomass. The protein-to-fiber ratio of leaves and press filtrate solids were 0.77 and 2.7, respectively. Protein-rich solids could have off-farm value, but their high water content necessitates anaerobic storage or drying. More work is necessary to determine how the functional and nutritional properties of ensiled protein affect its market potential.

Finally, this process would have less utility for production of lactic acid. Based on optimal conditions observed in our work, fermentation of the press filtrate could reach 143 kg ha^{-1} year^{-1} of lactic acid that, if purified to food grade, could be valued at USD $175 ha^{-1}. Unfortunately, purification has proven to be problematic, even with modern techniques. Therefore, it is our recommendation that lactic acid would be best utilized as an animal feed after protein solids have been recovered.

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