

Forage Handling, Preservation and Storage

Separation and Concentration of Soluble Protein from Plant Juice

R.G. Koegel, B.P. Lamsal, M.E. Boettcher, R.J. Straub

Introduction

Wet fractionation is the separation of fresh green herbage into a juice fraction and a high fiber fraction. In the case of alfalfa, the juice contains approximately 25% of the initial herbage dry matter and 35-40% of the herbage protein. This protein is in two forms: (1) particulate, green chloroplastic protein and (2) soluble, cream-colored cytoplasmic protein. The latter makes up approximately 40% of the juice protein or approximately 15-16% of the initial green herbage protein. This soluble protein is potentially food-grade with desirable functional properties (e.g. solubility, emulsification, foaming, etc.) In order to realize the potential high value of this protein it must be separated from the particulate fraction and concentrated without damaging functional properties.

Methods

The process of juice clarification by removal of the green particulates requires heating to 60C (142F) to aggregate particles, followed by centrifugation. This was carried out in a 15 cm (6 inch) diameter decanter centrifuge which provided 3000xG force. Since proteolysis takes place relatively rapidly, due to endogenous enzymes, it is desirable to quickly reduce juice temperature to near freezing as soon as possible and to hold it there for the remainder of processing. Protein concentration and removal of dissolved solids from the clarified juice was accomplished by ultrafiltration. A polysulfone membrane with 10,000 molecular weight cutoff was used to retain protein molecules while allowing smaller molecules and liquids to pass through the membrane. A dynamic filtration apparatus was designed and built. This apparatus has a 140 mm (5.5 inch) diameter rotor which can rotate up to 3000 rpm to create fluid shear forces for reducing retentate concentration on the membrane surface. The apparatus can be operated in two modes: (1) with the membrane attached to the surface of the rotor or (2) the membrane can be attached to the stationary surface separated by a small gap from the rotor surface. Typically the apparatus was operated with the outlets adjusted to give a permeate:retentate ratio of 4:1 giving a theoretical protein concentration of 5:1. A two level, three factorial experiment was conducted to determine the relative importance on flux (flow rate) of (1) rotor speed, (2) transmembrane pressure, and (3) gap between stationary and rotating surfaces. The two levels of the three variables used were: speed 1500,3000 rpm; pressure 25,50 psi, and gap 2,5mm.

Results

For the levels of variables chosen, the most important variable affecting flux was rotational speed, followed closely by transmembrane pressure. Contrary to expectations, gap between stationary and rotating surfaces had insignificant effect for the two levels of variable chosen. Since both rotational speed and gap contribute to shear rate which help to reduce buildup of retained materials on the

membrane, its lack of effect is surprising. Flux tends to decrease slowly with time. Typical flux values for clarified alfalfa juice generally ranged from 35-50 liters/sq. m/hr after two hours while initial flux with distilled water, by contrast, was 1100-1200 liters/sq. m/hr for relatively new membranes.

Conclusions

The flux observed for clarified alfalfa juice was consistent with that reported by other workers and is also consistent with that of similar proteinaceous materials such as whey. The importance of the gap variable needs to be evaluated for values outside the 2-5mm range. At a flux of 40 liters/sq. m/hr, it would require about 32 membranes of 1m diameter in parallel to achieve a flow through the membranes of one cubic meter or 1000 liters per hour. At an initial protein concentration of 1.5-2% in the clarified juice, this would result in 15-20 kg (33-44lb) of protein being concentrated to about 7.5-10%.

Production of Hydrolytic Enzymes by Solid Substrate Cultivation

R. G. Koegel, H.K. Sreenath, M.E. Boettcher, and R.J. Straub

Introduction

The fermentation of plant fiber (ligno-cellulosics) to ethanol, lactic acid, or other chemicals is a two step process. The first step is enzymatic depolymerization of the fiber to fermentable sugars which can then be fermented, as the second step, by an appropriate microorganism to the selected product(s). The two steps may be carried out concurrently in what is referred to as simultaneous saccharification and fermentation (SSF). While inexpensive raw materials, such as crop residues, can be converted to relatively valuable products in this way, the current high cost of commercially available enzymes makes the economics of these conversions dubious. For example, the US Department of Energy has estimated that the cost of enzymes needed for producing one gallon of ethanol from lignocellulosics to be around \$0.60. Enzymes are typically produced commercially by growing organisms in liquid culture in large aerated vats. The resulting enzymes must then be extracted from the dilute nutrient broth, concentrated, dried, and packaged for shipment to the point of use. It has been proposed that enzymes could be produced much more cheaply by cultivation of the microorganisms on solid substrate. One proposed scheme is to inoculate a small fraction of the fermentation substrate with aerobic fungi, known to be good enzyme producers under appropriate conditions. After the fungus had adequate time to multiply and to produce abundant enzymes, this fraction of the substrate would be mixed with the main portion as a source of the needed enzymes. No separation, concentration, drying, packaging, or shipping of the enzymes would take place. The total substrate would then be inoculated with the desired fermentation organism and conditions maintained to maximize fermentation while terminating growth of the enzyme-producing fungus. An analogous system has traditionally been used in Asia in the production of fermented rice products. Biopulping of wood to reduce the energy required for comminution is a related process. Difficulties which have been identified in scaleup of solid state cultivation of fungal enzymes to industrial scale include uniform aeration throughout the substrate mass and uniform removal of excess heat generated by the metabolism of the organisms. Pulsed pressure aeration has been advocated to overcome both problems.

Methods

A 20 liter pulsed aeration bioreactor was designed and built to address the problems of gas and heat transfer throughout the substrate. Pressure typically cycled between 0 and 15 psi gage with around 6 cycles per minute. Incoming air was bubbled through a heated water bath to achieve the desired temperature and humidity. Sterilized substrate was inoculated with the selected organism. The substrate was typically sampled and assayed at 24 hour intervals for enzyme activity. This was accomplished by washing the sample substrate and measuring enzyme activity in the filtered wash water. The two most frequently used organisms were *Aspergillus niger* and *Trichoderma reesei* RUT C-30, a mutant strain developed for cellulase production. The most frequently used substrate was alfalfa fiber resulting from wet fractionation. However, spent cellulose sausage casings were also used after shredding. In addition to the almost pure cellulose of the casing material, these also contained meat juices. Certain other trace nutrients were added to the substrates in various trials of fungal cultivation.

Results

Initially heat was added to the bioreactor by the inflow of air from the heated water bath. As the organisms multiplied, their metabolic activity added heat causing the substrate temperature to increase. Whenever either the substrate temperature or the water temperature exceeded the target temperature, the waterbath heater was automatically turned off until temperatures fell below the target. The substrate temperature was thus maintained between narrow limits while also maintaining moisture in the substrate. *Aspergillus niger* grew well on alfalfa fiber and other agricultural residues, appeared to out-compete any contaminants, and yielded high xylanase activity (in the range of 500-1000IU/g substrate dry matter). Results from *Trichoderma reesei* RUT C-30 were inconsistent. Adequate cellulase activity was sometimes achieved. However, on a number of occasions, either cellulase activity was low and/or the *Tricoderma* appeared to be inhibited by competing organisms. When growing well, however, it effectively hydrolyzed the cellulose sausage casings.

Conclusions

Solid substrate cultivation of organisms to produce enzymes for the saccharification of lignocellulosic feedstocks appears to have potential for reducing enzyme costs. The degree of success, to date, appears to depend on the robustness of the organism chosen. Therefore, additional work is required to identify the most appropriate organisms along with environmental and nutritional conditions which will allow the organisms to out-compete invading organisms while producing enzymes abundantly. Pulsed aeration showed potential for dealing with the dual problems of heat build-up and inadequate oxygenation as operations are scaled up.

Medium Density Fiberboard from Alfalfa Fiber

R.G. Koegel, R.J. Straub, and M.E. Boettcher

Introduction

A number of non-traditional products can be made from forage crops, like alfalfa, by means of wet fractionation. Wet fractionation consists of dividing herbage into juice and fiber fractions. Products from the juice fraction (about 25% of the initial dry matter) include feed-grade and food-grade protein concentrates, carotenoids, chlorophyll, and enzymes. Products from the fiber fraction (about 75% of the initial dry matter) include chemicals such as ethanol and lactic acid produced by fermentation, biofilters, fuels by means of gasification or direct combustion, and structural products such as fiberboard. Currently most fiberboard is made from wood fiber which is a byproduct of wood milling. It depends on synthetic, petroleum-based adhesives for its strength and integrity. This adhesive is also the major expense in fiberboard production. While it is doubtful that use of agro-based fibers could significantly reduce the cost of fiberboard, any reduction in the requirement of synthetic adhesive could make a contribution to decreased cost.

Methods

Fiberboard was made from alfalfa fiber obtained either from wet fractionation of alfalfa herbage or by washing feces from dairy cattle fed on a ration high in alfalfa (>90%). The adhesive used was alfalfa juice from wet fractionation. The mixture of fiber and juice was placed between 6 inch x 6 inch platens in a hydraulic press where pressures ranging from 200-400psi were applied while heating each platen with two 600 watt heaters from 350-450 deg.F. It was postulated that the proteins and carbohydrates in the juice in the presence of heat and moisture would undergo the Maillard reaction to form an insoluble complex which would act as an adhesive. The ratio of juice weight: fiber weight was in the range 0.5-1.0. Softening and flowing of the lignin in the fiber above 300 deg.F was thought to act as a second adhesive. The temperature was raised to the target value, held for approximately five minutes, heaters were turned off, and the temperatures were allowed to return to ambient. Samples were soaked in water for 24 hours and % increase in weight noted. Resulting materials were cut into rectangles and broken, in a testing machine, in beam bending to determine strength and rigidity.

Results

Initial results indicated that fiber washed from feces made board which was stronger and more stable than that made from fresh fiber. Removal of the more easily digested fiber components by the bovine digestive system appeared to reduce shrinking and swelling which lead to delamination. Therefore only manure-derived fiber was used in subsequent trials. Rifts or tears due to steam formation between the platens was a problem in materials with higher levels of juice. This was overcome by predrying the material to moisture contents below 20%. In materials with no juice added, strength was significantly lower and moisture absorption significantly higher, indicating that the “cementing” due to the thermal softening of lignin played a secondary role. Densities of the board generally exceeded 50 lb/cu ft which ranked it as “high density”. The most stable boards (400 psi, 450 deg.F, fiber:juice=1.0:0.67) increased in weight by 8-13% during 24 hours of soaking. The strongest board had a modulus of rupture ranging from 21-26 Mpa (3100-3800psi) just sufficient to meet the requirements of “medium density” while the strength of most boards ranked as “low density” or

below. The modulus of elasticity of the most stable boards exceeded the requirement for “medium density” board (2400 Mpa) and frequently exceeded requirements for “high density” board. According to US Patent 5,371,194, the integrity of board with this type of adhesive can be improved by ammoniation to alkalinity prior to pressing. This effect could not be detected, however.

Conclusions

Alfalfa fiber, washed from bovine feces, and mixed with alfalfa juice at a ratio of around 1: 0.67, made medium density fiberboard of good integrity when pressed at 450 deg.F and 400 psi. The modulus of rupture of this board was marginal relative to the ANSI requirement, however. Modest addition of synthetic adhesives should be tried to ascertain if strength could be augmented to meet or exceed ANSI standards at nominal cost.

Ohmic Heater for Treatment of Plant Juice

R.G. Koegel, R.J. Straub, M.E. Boettcher

Introduction

Rapid, uniform heating of plant juice is an important step in the removal of particulate (chloroplastic) protein. Heating with conventional heat exchangers is problematic due to localized overheating and to fouling of heat exchanger surfaces. Direct steam injection into the juice has been used to overcome these problems. Localized, instantaneous overheating can still result and the steam concentrate dilutes the juice. Since the juice has high electric conductivity, it is possible to heat it by direct passage of electric current through the juice. It has been claimed by workers in the former Soviet Union that the passage of alternating current through the juice also ruptures any intact chloroplast membranes freeing soluble protein held within the membrane.

Methods

A batch type heater was designed and built which used three-phase, 208 volt or 480 volt alternating current (Fig. 1). The electricity was conducted to and from the juice via three graphite paddles equally spaced on a vertical shaft rotor. Slip rings provided a path from the electrical source to the rotor. The rotor was driven by a three-phase electric gear motor equipped with a variable frequency speed control to allow varying the rotational speed. It was typically run at 30-50 rpm. Rotation served dual purposes of stirring the juice to keep the temperature uniform throughout while avoiding any buildup on the paddles. The capacity of the juice container was 55 gallons (~200 liters) The three paddles each had an area of 36 sq. inches (~230 sq. cm) and were at a radius of 8 inches (20cm) from the rotor axis.

Results

As observed in earlier research, juice conductivity, and thus current flow, increase as temperature increases. Juice was generally heated from around 65F (18C) to 131F (55C). Batch size did not generally exceed 20 gallons (77 liters). Initially the heater was run on 480 volts which led to a current flow exceeding 50 amps for a calculated power of around 42 kw. This resulted in the juice

temperature being raised approximately 65F (36C) in about four minutes. Because the current, at 480 volts, tended to exceed the 50 amp rating of the circuit used, the heater was subsequently run on 208 volts. At this voltage the current was about 20 amps and the heating time around 15 minutes. No fouling of the graphite electrodes was observed over more than a month of use. At the higher current flow of 50 amps, current density on the electrodes was about 0.2 amps/ sq. cm. No attempt was made to verify whether soluble protein was augmented by rupture of chloroplast membranes.

Conclusions

The ohmic heating apparatus functioned as intended. Lack of fouling at the electrodes indicates absence of localized overheating. Reducing the time that juice is at elevated temperatures is desirable to minimize proteolysis caused by endogenous enzymes. Therefore, running the heater at 480 volts would be an advantage. This should reduce heating time to roughly 20% of that for 208 volts. Evaluation of whether the alternating current electrical treatment increases availability of soluble protein by rupturing intact chloroplast membranes should be carried out.

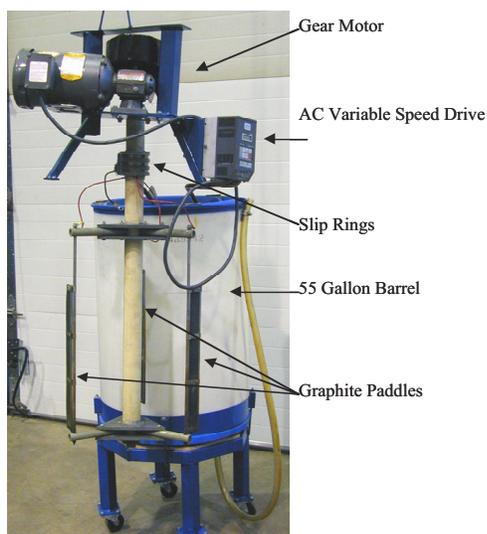


Fig. 1: Electrical Juice Heater

Inoculant Effects on Aerobic Stability of Corn Silage

R.E. Muck

Introduction

Inoculants are the most common additives used in making silage in the U.S. These products provide selected lactic acid bacteria to supplement the natural population of lactic acid bacteria on the crop and ensure a rapid and efficient silage fermentation. While these products have provided improvements in dry matter recovery and animal performance, aerobic stability (the time until the silage begins to heat during feed out) has sometimes been made worse by inoculants, particularly in corn and other whole-crop grain silages. Inoculant manufacturers are aware of this problem and have been working on developing inoculants that more consistently improve aerobic stability. The objective of this study was to test several new types of inoculants and compare their effects on aerobic stability with current products.

Methods

Whole-crop corn was harvested with a forage harvester in each of three years. The chopped corn was ensiled in 60 x 10 cm dia. PVC silos sealed with a rubber end cap on one end and with black plastic secured with duct tape on the other. The number of treatments varied from year to year depending on product availability, but most of the products were tested all three years. Treatments included an uninoculated control, three standard corn silage inoculants available in the market, a new product with improved homofermentative strains, several new products with the heterofermentative species *Lactobacillus buchneri*, and a prototype product (1 year only) with a standard inoculant plus a chemical spoilage inhibitor. All products were applied at recommended rates but were diluted with water such that each treatment was applied at 1 g/50 g crop. The control received 1 g water/50 g crop. The silos were opened after a minimum of 90 days ensiling. Silos were weighed prior to emptying. The spoiled silage on the top was removed and weighed. The rest of the silage was removed, mixed and analyzed for microbial groups, pH, fermentation products and moisture content. The remainder was placed in Styrofoam buckets, and silage temperatures were recorded hourly until heating occurred.

Results and Discussion

The pHs and aerobic stabilities of the silages in all three years are summarized in Table 1. The standard inoculants and the improved standard inoculant had no effect on silage pH relative to the control in any year; all had excellent pHs of 3.62 to 3.90. The *L. buchneri* inoculants raised pHs significantly from the control, typically 0.1 to 0.3 units. The cause of the higher pH was a shift in fermentation – increased acetic acid and ethanol concentrations, reduced lactic acid concentrations.

The *L. buchneri* inoculants produced the most consistent improvements in aerobic stability across the three years. Only in year 2 with *L. buchneri* 3 was the improvement in aerobic stability not statistically significant ($p < 0.05$). The standard inoculant plus chemical spoilage inhibitor was tested in only one year but provided a 3-day improvement in aerobic stability relative to the uninoculated control. The standard inoculants generally had trends toward reduced aerobic stability, as expected, although there was only one significant effect (a reduction in stability in year 3 by Standard 1). The improved standard inoculant was similar to the standard inoculants in the first two

years but provided significantly greater stability than the control in year 3. Aerobic stability across the treatments was negatively correlated with yeast counts as indicated in Fig. 1. No other factors were well correlated with aerobic stability. These results suggest that the primary means of improving aerobic stability in the effective products were by lowering yeast populations, the frequent initiators of heating in corn silage.

The standard inoculants and the improved standard inoculant generally had the lowest dry matter losses although trends were not statistically significant. The *L. buchneri* products typically had dry matter losses numerically between the standard inoculants and the control. Experience in these trials and earlier ones with *L. buchneri* suggest that dry matter recoveries are approximately one percentage point less than those with standard inoculants because of the shift to more heterofermentative products with *L. buchneri*.

Overall, the *L. buchneri* inoculants were more consistent than the improved standard inoculant in enhancing aerobic stability. This came with a small cost to dry matter recovery. While these trials addressed fermentation and aerobic stability, the principal return to the farmer from using inoculants has been in improved animal performance. Animal research trials with the *L. buchneri* products are beginning to be published. These have found similar improvements in aerobic stability but have yet to show improved animal performance compared to that from uninoculated silage.

Conclusions

Of the new corn silage inoculants available to farmers, the *L. buchneri* inoculants provided the most consistent improvement in aerobic stability. An improved standard inoculant was better than standard inoculants in one year of three relative to aerobic stability. At this stage of testing, selection of a corn silage inoculant appears to hinge on the most important goal(s) of the farmer. If poor aerobic stability in corn silage and its effect on animal performance are consistent problems that have not been solved by improved silo management, then the *L. buchneri* products show the most promise. However, if the primary goals are improved animal performance and dry matter recovery, then the improved standard and conventional homofermentative inoculants are more likely to achieve success.

Table 1. Characteristics of the silages.

Inoculant	pH			Aerobic Stability Relative to Control, h		
	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
None (Control)	3.82	3.87	3.64	0	0	0
Standard 1	3.85	3.89	3.71	16	-13	-40
Standard 2	3.84	3.90	3.65	-4	-20	-6
Standard 3	3.83	3.90	3.62	-25	-6	-10
Improved Standard	3.81	3.90	3.64	-24	-27	29
Standard + Inhibitor	3.83	—	—	76	—	—
<i>L. buchneri</i> 1	4.01	4.11	4.01	142	100	811
<i>L. buchneri</i> 2/3*	3.90	4.06	3.84	103	22	454

* *L. buchneri* 2 in year 1; *L. buchneri* 3 in subsequent years.

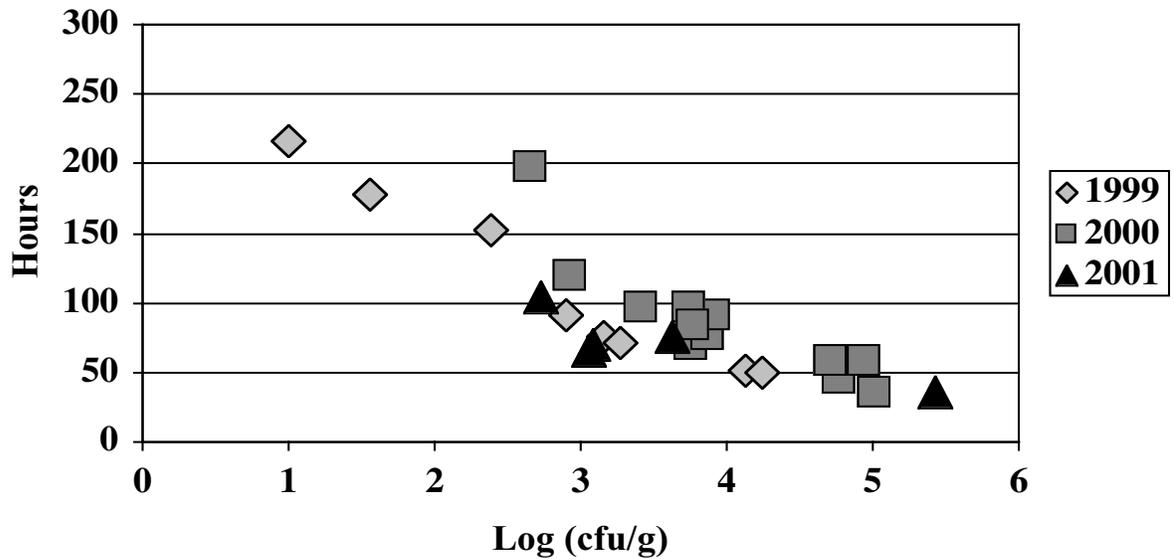


Fig. 1. Aerobic stability of the treated silages as correlated with yeast counts in the silages at opening. A yeast count of 1 log(colony-forming units/g crop) indicates that all four silages in the treatment were below detectable level (100 cfu/g crop).

Density and Losses in Pressed Bag Silos

R.E. Muck and B.J. Holmes

Introduction

The pressed bag silo is an increasingly popular method of making silage. It is relatively inexpensive. Storage size varies with the quantity of forage harvested. For farms that are expanding in herd size, silo capacity can be added with little capital cost. Small diameter bags allow small farms to consider making silage rather than hay. Finally, bag silos make it easy for farmers to inventory and manage silage, e.g., reserving high quality silage for the best animals.

Unfortunately there are limited data on the densities and losses from bag silos. This makes accurate economic assessment of bag silos difficult for farmers considering using them. It also hampers farmers with bag silos in assessing their silage inventory.

Methods

We monitored the filling and emptying of bags at our research farm at Prairie du Sac and two University of Wisconsin Agricultural Research Station farms (Arlington, West Madison) over the 2000 harvest season. Most of the silages were alfalfa or whole-plant corn. All loads of forage entering the bags were weighed and a sample taken for analysis. After each load was pressed into the bag, the side of the bag was marked to indicate the distance filled by the load. Each load sample was

analyzed for moisture content. The remainders of samples were composited by field and date and analyzed for particle size distribution, crude protein, neutral detergent fiber, moisture and ash.

At emptying, the weight of all silage removed from a bag was recorded. Any spoiled silage not fed was weighed and specifically identified as such on the emptying log. A grab sample from the face of each silo was taken periodically, one per filling load. Samples from emptying were analyzed for pH and fermentation products in addition to those performed on the load samples. However, the samples from emptying were not analyzed for particle size distribution. Densities for the bags were calculated based on the weight ensiled, length and nominal bag diameter.

Results and Discussion

Over the course of the 2000 harvest season, a total of 25 bag silos were made at the three farms. The average dry matter densities of all the bags are shown in Figures 1 and 2. Dry matter (DM) density increased the drier the crop at ensiling, 2.9 and 5.3 kg/m³-% DM for alfalfa and corn silages respectively. Estimating densities at a constant DM content (40%), DM densities in alfalfa silages were approximately 200 kg/m³. Densities in corn silage were 3 to 8% lower than those in alfalfa with one bagging machine whereas densities were 16 to 35% higher in the other bagging machine. One bagging machine was shared between two farms, and one farm consistently achieved a higher density (approximately 10%) than the other, indicating the operator affects density.

So far 15 bags have been completely emptied and results analyzed. Average DM losses were 8.4% gaseous/seepage loss (i.e., weight loss out vs. in) and 5.8% spoilage loss (i.e., silage removed from the bag but not fed) for a total of 14.2% loss. The average spoilage and total losses were inflated by three bags with substantial spoilage (26 to 38% total loss). One of those bags sustained major bird damage on the top that was not noticed immediately and repaired. In contrast, eight bags had no spoilage loss or very minor spoilage at the ends. Removing the three bad bags from the average reduced average total losses to 9.7%. Gaseous losses increased with low feed out rates (<30 cm/day) whereas spoilage losses were associated with drier (>40% DM), more porous silages. Overall, these results suggest that DM losses similar to those in tower silos are achievable with good silo bag management.

Conclusions

Densities in bag silos were affected by crop DM content, crop type, bagging machine and how the operator set up the bagging machine. At 40% DM, DM densities in alfalfa averaged 200 kg/m³. Densities were higher in corn silage with one machine and lower in the other. DM losses in bag silos can be similar to those in tower silos (i.e., less than 10%), but large losses (>25%) can occur if management is less than ideal. Losses will be minimized by ensiling between 30 and 40% DM, routinely monitoring for and patching holes, and feeding out at least 30 cm/day from the face.

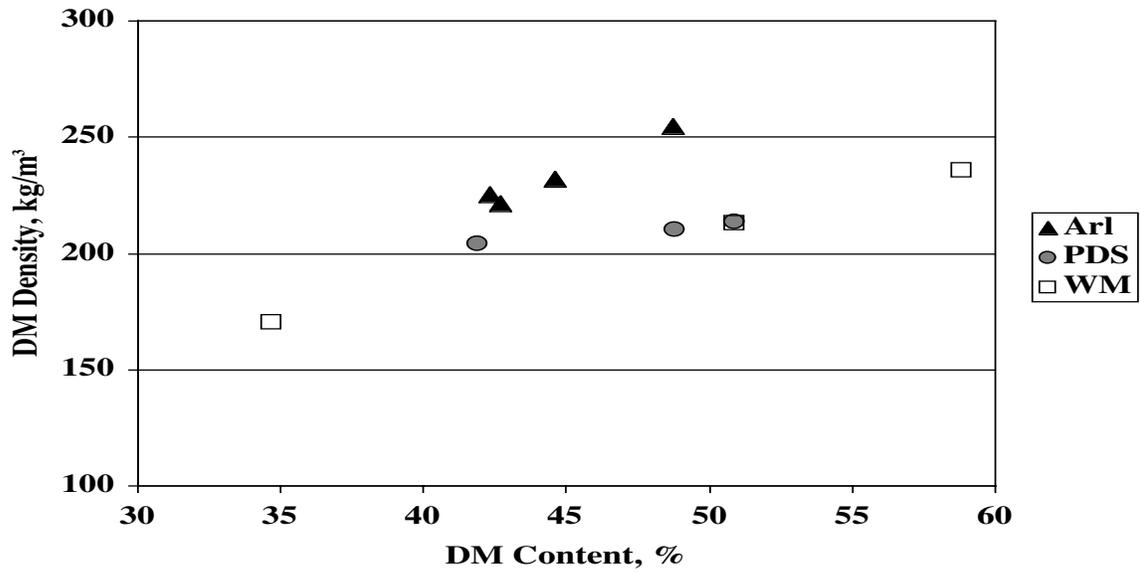


Fig. 1. Average dry matter densities of hay crop silages made in bag silos at different dry matter contents and farms (Arl – Arlington, PDS – Prairie du Sac, WM – West Madison).

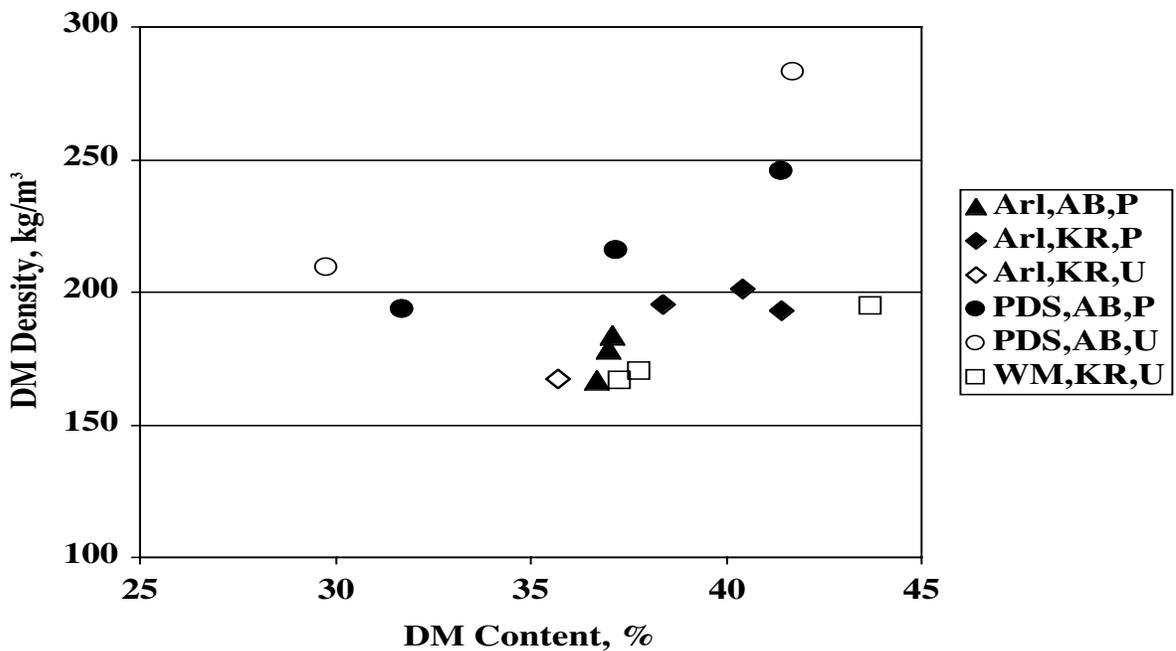


Fig. 2. Average dry matter densities of corn silages made in bag silos at different dry matter contents, farms (Arl – Arlington, PDS – Prairie du Sac, WM – West Madison), machines (AB – Ag Bag, KR – Kelly-Ryan) and kernel processing (P – processed, U – unprocessed).