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Reprinted from AGRONOMY JOURNAL
Vol. 60, Nov.-Dec. 1968, p. 658-659

Effect of Drying Methods on Losses of Carbon, Nitrogen and Dry Matter From Alfalfa[1]

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ABSTRACT

Methods of drying forage samples were examined for their influence on the total C, N, and dry matter (DM) content of 'Ranger' alfalfa (*Medicago sativa* L.). Forage samples were freeze-dried; air-dried in perforated paper bags and on trays; dried in perforated paper bags in mechanical convection ovens at 60, 80, and 100 C; and oven-dried at 100 C for 90 minutes followed by drying at 60 C, or by air-drying. All treatments were terminated after 48 hours.

Losses in C and N were measured by changes in C:K and N:K ratios. The DM losses were measured by changes in K concentration. Carbon and DM were lost more readily than N by either respiratory or thermochemical processes. These losses resulted in an apparent concentration of N in all forage dried between ambient and 100 C. All treatments, when compared to freeze-drying, resulted in losses of C and DM. The greatest loss of DM, 5.4% occurred in samples dried at 100 C. Oven-drying at 100 C for 90 minutes followed by air-drying caused DM losses of only 0.2%. Significant differences in actual N content between the various drying methods were not generally observed.

Additional index words: **freeze-drying, oven-drying, lyophilization, potassium.**

THE main objetcive in drying forage samples before analysis is to provide a reproducible basis for expressing the physical and chemical qualities. Some drying techniques result in dry matter (DM) losses, which are not easily separated from losses of water and are consequently included in the measurement of the water content of the sample (3).

Drying between 0 and 30 C results in degradation of sugars (4) and subsequent losses of C and DM. Such losses are proportional to water content and result from continued enzymatic respiration during drying (4, 5). Dry matter losses at temepratures above ambient result from degradation and volatilization of cellular constituents, and are proportional to temperature (5, 6, 7).

Some commonly used drying methods alter or even destroy certain chemical constituents (1, 4, 5). Burns et al. (1) state, "Since the method of drying had a definite effect on the total and water soluble nitrogen constituents of forage plants, analyses on oven-dried samples may not be representative of green plant material."

The objective of this study was to measure the actual losses of C, N, and DM in alfalfa forage dried by several methods.

MATERIALS AND METHODS

Second-cutting 'Ranger' alfalfa (*Medicago sativa* L.) was clipped at the prebloom stage, mixed, chopped into 0.5- to 1-cm lengths, and again mixed. Subsamples (150-175 g wet wt., 22% DM) were assigned randomly to treatment and one of 4 replications. Drying treatments began 45 minutes after harvest. Respiratory losses during this 45-minute period were not measured.

Forage was dried by lyophilization (freeze-drying); air-drying at ambient temperatures (ca. 23 C); or drying in forced-draft ovens at temperatures of 60, 80 or 100 C. Freeze-dried samples were frozen with dry ice prior to drying. Samples to be oven-dried were placed loosely in open, 25-pound paper hardware bags having about 50 perforations 2 mm in diameter. Two additional treatments were designed to differentiate between enzymatic and thermochemical losses of C, N, and DM. These treatments involved drying at 100 C for 90 minutes followed by continued oven-drying at 60 C or air-drying on 36- × 46-cm trays. All drying treatments continued for 48 hours.

Samples were ground immediately after drying to pass a 60-mesh sieve. The residual water content of samples was determined by drying subsamples to constant weight in evacuated desiccators containing P_2O_5. The ground material reserved for chemical analyses was placed in open weighing dishes and stored in desiccators containing P_2O_5 at 22 C and reduced pressure.

Carbon was determined gravimetrically after dry combustion of 0.1-g samples at 900 C in the presence of excess O_2 for a period of 7 minutes. The O_2 stream flowed at a rate of 10 liters per minute and was prescrubbed of CO_2 and H_2O by passing through $Mg(ClO_4)_2$ and Ascarite.[3] After passing over the ignited sample, the gas stream was scrubbed of halides and H_2O by passing it through saturated Ag_2SO_4 and $Mg(ClO_4)_2$ traps. The CO_2 resulting from ignition of the plant C was trapped on Ascarite and weighed. Nitrogen was determined by the Kjeldahl method with pretreatment to include NO^-_3 (2). Ash was determined gravimetrically after dry ignition of 2 g of plant material at 600 C for 4 hours. The ash was dissolved with 5 ml 6 *N* HCl, evaporated to dryness, diluted with H_2O, and filtered to remove silica. Potassium in the dissolved ash was determined with a flame photometer.

RESULTS AND DISCUSSION

The concentration of plant constituents is normally reported on a dry-matter basis. Because DM is lost during some drying procedures, it cannot provide a reliable basis for determining small concentration differences in plant constituents resulting from different drying techniques. Metallic elements are not lost by drying and careful ashing. Therefore, total ash and K concentration were selected as the basis for determining changes in plant constituents as affected by drying method. The coefficient of variation for total ash analyses was 1 to 3%; for K analyses, 0.1 to 0.3%. Analysis of variance of K:ash ratios showed no significant differences among drying methods. Potassium content of the dried forage sample was used as the reference for determining DM losses and the C:K and N:K ratios were used to determine C and N losses.

Percentages of C (Table 1) determined on the basis of DM were remarkably similar, implying that method of drying had little effect on C losses. However, the C:K ratios and K percentages did not vary in the same way as C percentages, suggesting that losses of C and other elements such as N and O were not proportional in all treatments. The C:K ratio was highest and K

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[3] Mention of a proprietary product does not necessarily imply endorsement of this product by USDA.

Table 1. Percentages of residual H_2O, C, N, and K relative to dry matter content; respective elemental ratios; and dry matter losses based on K concentration in dried alfalfa as affected by drying method.

Drying treatment	Treatment means*							% dry matter losses
	% H_2O	% C	% N	% K	C:N†	C:K†	N:K†	
Freeze dry	3.46 d	44.43 cd	3.032 a	2.348 a	14.66 c	18.93 c	1.291 ab	0.0 a
100 C (90 min) 60 C	2.73 g	44.15 a	3.137 bcd	2.414 bc	14.08 ab	18.30 abc	1.300 ab	2.8 bc
100 C (90 min) air dry	3.64 c	44.25 abc	3.127 bcd	2.353 ab	14.16 abc	18.82 bc	1.329 b	0.2 ab
100 C	2.41 h	44.79 e	3.076 ab	2.476 c	14.56 bc	18.09 ab	1.242 a	5.4 c
80 C	2.92 f	44.59 de	3.113 bc	2.457 c	14.32 abc	18.16 abc	1.268 ab	4.6 c
60 C	3.29 e	44.39 bcd	3.174 cd	2.457 c	13.99 a	18.08 ab	1.296 ab	4.6 c
Air dry on trays	3.95 a	44.59 de	3.135 bcd	2.443 c	14.23 abc	18.27 abc	1.284 ab	4.0 c
Air dry in bags	3.70 b	44.17 ab	3.205 d	2.464 c	13.78 a	17.93 a	1.301 ab	4.9 c

* Data in a column followed by the same letter are not significantly different at 5% probability. † Data represent means of individual sample ratios.

percentage was lowest in the freeze-dried alfalfa. Carbon losses were as high as 5.3% as determined from the C:K ratios.

On a dry-matter basis, the N percentage of alfalfa appeared to be smaller for freeze-drying than for any other treatment. Based on the N:K ratio, alfalfa dried at 100 C for 90 minutes followed by air-drying lost significantly less N than alfalfa dried at 100 C for 48 hours. The N content (based on N:K ratio) of samples dried by these two methods, however, was not different from that of samples dried by the other methods.

The DM losses were as high as 5.4%, based on K content. With the exception of the freeze-drying treatment and heating to 100 C for 90 minutes and then air-drying, all other treatments effected DM losses which were not significantly different.

Losses of C and DM occur by enzymatic or thermochemical degradation. Enzymatic loss of DM is a function of both drying temperature and moisture content of the forage, and continues to about 60 C or until moisture becomes limiting. Enzymatic losses of DM were highest during air-drying of alfalfa on trays or in bags, resulting in losses of 4.0 and 4.9% respectively. Thermal loss was highest during drying at 100 C. However, a mass of fresh forage placed in an oven does not reach oven temperatures for a period of time, and during that time undergoes moist incubation and enzymatic respiration. The DM loss of 5.4% resulting from oven-drying at 100 C was a combination of respiration and thermochemical degradation.

Oven-drying at 100 C for 90 minutes destroyed the enzymes. Further drying at ambient air temperatures resulted in very small losses (0.2% DM), but continued oven-drying at 60 C resulted in 2.8% loss from thermochemical degradation. The 4.6% DM loss by samples dried at 60 C represents both enzymatic and thermal processes and illustrates the consequences of delayed or decreased drying rates.

CONCLUSIONS

Dry matter loss may be measured by concentration of mineral elements such as K. This element exists in relatively high concentration (2%) in some plant material and is measured easily and precisely. Sample ash might also be used as a base, but was measured less precisely than K in this study.

Water loss measurements obtained by oven-drying alfalfa would be too high because of associated DM losses of 2 to 5%. The residual water content of alfalfa samples was determined as the loss in weight over evacuated P_2O_5 and the amount was found to be significantly different among the drying methods compared.

Actual losses up to 5.3% C were not reflected in the C-percentage values. Losses of N were not proportional to those of either C or DM and resulted in an apparent increase in N percentage in samples dried at ambient or elevated temperatures. The actual N content of samples was significantly lower for samples dried at 100 C than for samples dried at 100 C for 90 minutes followed by air-drying. With the above exception, the N content was not significantly affected by the drying treatment.

Dry matter losses in alfalfa occurred when drying at ambient or higher temperatures. These losses were mainly from enzymatic respiration below 60 C and from thermochemical degradation at higher temperatures. A desirable technique for minimizing DM losses inactivates or destroys enzymes by high temperature or chemical means followed by drying at temperatures which do not promote thermal losses. Drying in a forced-draft oven at 100 C for 90 minutes, followed by air-drying, provides such conditions. Freeze-drying also inactivates enzymes by exposure to subzero temperatures during the lyophilization process. Special care, however, must be given to freeze-dried samples to prevent rehydration and subsequent reactivation of enzymatic respiration. The selection of a drying technique will depend on the accuracy and precision required for measuring plant components and the availability of drying equipment.

LITERATURE CITED

1. Burns, J. C., C. H. Noller, and C. L. Rhykerd. 1966. Influence of drying method and fertility treatments on the total and water soluble nitrogen contents of alfalfa. Agron. J. 58:13-16.
2. Chapman, H. D. and P. F. Pratt. 1961. Methods of analysis for soils, plants, and waters. Univ. Calif., Div. Agr. Sci. p. 150-152.
3. Greenhill, W. L. 1960. Determination of the dry weight of herbage by drying methods. J. Brit. Grassld. Soc. 15:48-54.
4. Melvin, J. F. 1963. Storage of hay. IV. Effect of storage in nitrogen on the soluble sugar and dry matter contents of ryegrass. J. Sci. Food Agric. 14:281-283.
5. Raguse, C. A. and D. Smith. 1965. Carbohydrate content in alfalfa herbage as influenced by methods of drying. J. Agr. Food Chem. 13:306-309.
6. Raymond, W. F. and C. E. Harris. 1954. The laboratory drying of herbage and faeces and dry matter losses possible during drying. J. Brit. Grassld. Soc. 9:119-130.
7. Wilkinson, R. and C. W. Hall. 1965. Respiration rate of harvested forage. Michigan State Univ. Agr. Exp. Sta. Quart. Bul. 47:518-526.