



Sample Preparation Affects Non Structural Carbohydrates in Alfalfa

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Introduction Non structural carbohydrate (NSC) concentration in forages provides an estimate of the energy readily available to rumen microbes. Proper sample preparation is crucial for precise estimation of NSC. Enzymatic activity that occurs after cutting may affect the final NSC concentration. Liquid N and freeze-drying are useful methods to quickly stop the enzymatic activity but they are difficult to use with large samples under field conditions. Other methods used for larger samples exist but their effectiveness remains to be determined. Our objective was to assess the effect of sample preparation treatments on the NSC concentration in alfalfa. We hypothesized that high temperature or freezing, immediately after harvest, will limit enzymatic activity and preserve NSC in alfalfa samples.

Materials and methods The experiment was conducted on a spring growth in 2006 and a spring growth and a summer regrowth in 2007. Field-grown alfalfa (around 8 kg) was cut, separated in 5 replication sets of 5 sub-samples of 250 g each, put on ice in 5 coolers, and kept in a refrigerator at 4 °C. As soon as possible after harvest (< 1 hr), the first set of 5 sub-samples of 250 g each was treated as follows: 1-frozen in a plastic bag at -20°C for approximately 1 month, and then freeze-dried (Freeze-drying), 2-heated in a microwave oven during 1 min at maximum intensity to reach approximately 70 °C and then dried at 55 °C for 48 hours (Microwave oven/Drying 55), 3-dried at 100 °C for 1 hour and then at 55 °C for 48 hours (Drying 100/55), 4-dried at 55 °C for 48 hours (Drying 55), and 5-frozen in a plastic bag at -20 °C for approximately 1 month, and dried at 55 °C for 48 hours (Freezing/Drying 55). The 4 other replication sets of 5 sub-samples were subsequently treated in the same way. Drying at 55 or 100 °C was done in a forced-air oven. Treatments 3 and 5 were not applied in 2006. Dried samples were ground using a Wiley mill (1-mm). Ground samples were analyzed for soluble carbohydrates and starch by a colorimetric method, and for pinitol by HPLC as previously described (Bertrand et al., 2003). Concentrations of NSC were estimated by the sum of soluble sugars, pinitol, and starch. For each harvest, the analysis of variance was performed using the MIXED procedure with the LSMEANS statement and the DIFF option of SAS to compare treatment means and calculate the standard errors of the means (SEM).

Results and discussion Drying forage samples at 55 °C for 48 hours resulted in the lowest NSC concentration at the three harvests (Table 1). Heating samples in a microwave oven for one minute followed by drying at 55 °C for 48 hours, however, resulted in similar or higher NSC concentrations than freeze-drying. Drying at 100 °C for 1 hour and then at 55 °C for 48 hours, or freezing at -20 °C for 1 month followed by drying at 55 °C for 48 hours gave similar results than freeze-drying.

Table 1 Non structural carbohydrates (NSC, mg/g dry matter) in alfalfa spring growth and summer regrowth harvested in 2006 and 2007 and dried in different ways.

Alfalfa	Sample preparation treatment					SEM
	Freeze-drying	Microwave oven/Drying 55	Drying 100/55	Drying 55	Freezing/Drying 55	
2006 Spring growth	149.0 a	161.8 a	-	91.7 b	-	8.02
2007 Spring growth	96.8 ab	97.1 ab	90.1 b	59.8 c	107.4 a	7.36
2007 Summer regrowth	76.9 b	90.9 a	72.2 b	57.0 c	72.8 b	6.50

In the same row, means followed by different letters are different at $P \leq 0.05$.

Conclusions Standard procedure of drying forage samples at 55 °C for 48 hours reduced the NSC concentration of alfalfa. High temperature treatment immediately after harvest, either with a microwave oven (1 min to reach 70°C) or with a draft oven (100 °C for 1 hour), helped prevent the loss of NSC in alfalfa samples. Freezing (-20°C) immediately after harvest also reduced this loss. High temperature and freezing treatments, however, may affect other characteristics of nutritive value; this remains to be determined.

Reference

Bertrand, A., Castonguay, Y., Nadeau, P., Laberge, S., Michaud, R., Bélanger, G., & Rochette, P. 2003. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. *J. Exp. Bot.* 54 : 1721-1730.