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Case Study: Fermentation of frozen whole-plant corn silage and high-moisture corn after thawing

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ABSTRACT

The study objectives were to evaluate (1) the effect of thawing unfermented whole-plant corn (WPC; Exp. 1) on fermentation capacity after several months of frozen storage, and (2) the effect of temperature on fermentation profile of thawed high-moisture corn (HMC; Exp. 2) stored frozen for a longer period and then fermented at 2 ambient temperatures. Unfermented WPC and HMC samples were obtained from the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) at harvest, immediately frozen, and stored at −20°C for 4 or 17 mo, respectively. Both, WPC and HMC samples were thawed, homogenized, and divided into 24 or 33 subsamples, respectively. Subsamples were vacuum sealed in plastic bags and randomly assigned to treatments with 3 replications each. Experiment 1 treatments received 0, 0.5, 1, 2, 3, 7, 14, and 28 d of fermentation. Experiment 2 treatments were mini-silos fermenting in the dark either at warm (at room temperature 20°C; WR) or cold (in the refrigerator set for 3°C; CD) temperatures and allowed to ferment for 1, 3, 7, 14, or 28 d, plus 3 random subsamples analyzed as fresh samples. Gradual increases in lactate and acetate concentrations were observed in Exp. 1, along with a gradual decrease in pH ($P = 0.001$). A temperature × ensiling time interaction was observed ($P < 0.001$) in Exp. 2 for all fermentation profile measurements. This was related to fermentation occurring in WR but not in CD treatments. These findings suggest that WPC and HMC maintain fermentation capacity upon thawing even after being frozen for a prolonged period in storage, but fermentation will not occur until warm temperature is reached.

Key words: corn silage, high-moisture corn, fermentation, frozen silage

INTRODUCTION

Ensiled whole-plant corn silage (**WPC**) and high-moisture corn (**HMC**) are widely used in diets for dairy cows. Late harvest of WPC and HMC into late fall and winter months during 2014/2015 raised concerns among central and northern Wisconsin dairy farmers and their nutritionists about fermentation of frozen WPC and HMC. Fermentation is a key aspect of silage preservation (McDonald et al., 1991), and it was recently suggested as a tool to increase starch digestibility through the breakdown of prolamin proteins surrounding starch granules (Hoffman et al., 2011). However, several management practices, including temperature, affect the microbial population and, thereby, fermentation in silage during storage (McDonald et al., 1991). Although research trials on the effects of high temperatures at ensiling on silage quality are available in the literature (Weinberg et al., 2001; Kim and Adesogan, 2006), research focused on low temperature is limited (Wang and Nishino, 2013; Zhou et al., 2016), and upon thawing, to our knowledge, research is unavailable.

Therefore, the study objectives were to evaluate (1) the effect of thawing unfermented WPC (Exp. 1) on fermentation capacity after several months of frozen storage, and (2) the effect of temperature on fermentation profile of thawed HMC (Exp. 2) stored frozen for a longer period and then fermented at 2 ambient temperatures. We hypothesized that silage would ferment upon thawing, but the extent of fermentation would be reduced under low temperatures.

MATERIALS AND METHODS

Exp. 1

An unfermented WPC sample was obtained at harvest from the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) on September 23, 2014, immediately frozen, and stored at −20°C until January 26, 2015. Sample was thawed in a refrigerator set for 3°C, homogenized, and allocated into 24 subsamples of approximately 300 g each using a quartering technique: homogeneous samples were divided into 4 equal subsamples. Two subsamples were saved for later treatment, whereas the 2 other subsamples were rehomogenized and redivided; the process was repeated until 24 subsamples of approximately 300 g were prepared. The remainder (fresh sample) was frozen at −20°C until being processed for analysis to characterize the material. Subsamples were allocated

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Table 1. Descriptive statistics for unfermented whole-plant corn and high-moisture corn dry matter content and physical characteristics

Item	Mean	SD
Whole-plant corn		
DM, % of as fed	38.7	5.8
Penn State separator sieves,[1] % as fed retained		
19.0 mm	18.1	10.2
8.0 mm	62.6	8.4
1.18 mm	18.2	2.4
Bottom pan	1.1	0.6
Processing score,[2] % passing a 4.75-mm sieve		
Starch	50.2	6.1
High-moisture corn		
DM, % of as fed	70.7	0.8
Mean particle size, μm	1,404	39
Surface area, cm^2/g	31	3

[1]Particle size was measured using the Penn State particle size separator as described by Kononoff et al. (2003).

[2]Corn silage processing score was measured as described by Ferreira and Mertens (2005).

in nylon-polyethylene standard barrier vacuum pouches (89-μm thickness, 25.4 × 35.6 cm; Doug Care Equipment Inc., Springville, CA), vacuum heat sealed using an external clamp vacuum machine (Bestvac; distributed by Doug Care Equipment Inc.), and randomly assigned to 8 treatments so that each treatment had 3 replications. Treatments were 0, 0.5, 1, 2, 3, 7, 14, and 28 d of fermentation. Bags were stored in the dark at room temperature (approximately 20°C) until the targeted ensiling time was reached.

The fresh sample was analyzed undried and unground for particle size as described by Kononoff et al. (2003), whereas dried (at 60°C for 48 h in a forced-air oven) and unground samples were analyzed for corn silage processing score (Ferreira and Mertens, 2005) at the University of Wisconsin–Madison for material characterization (Table 1). All ensiled samples (including 0 d) were analyzed for DM, pH, organic acids, and ammonia-N (% of DM) at Rock River Laboratory Inc. (Watertown, WI). Content of DM was determined by drying samples at 105°C for 3 h in a forced-air oven (NFTA, 1993; method 2.2.2.5). For organic acids analysis, 20 g of undried and unground sample was diluted 10-fold (mass basis) in double distilled water, blended for 30 s in a high-speed blender, and filtered through a filter funnel with a 2-mm filter screen. The extract was collected and analyzed for pH using a pH meter (Thermo-Orion Dual Star; Thermo Fisher Scientific Inc., Waltham, MA) fitted with a glass pH electrode (Thermo-Orion 9172BNWP; Thermo Fisher Scientific Inc.). After pH was measured, the extract was centrifuged (750 × *g*) for 30 min at 25°C, and the supernatant was combined with calcium hydroxide and copper sulfate and recentrifuged as described previously. Supernatant was analyzed for organic acids using HPLC with isocratic pump, auto sampler, column heater, and refractive index detector (Waters Corporation 1515, 2707, Heater, and 2414, respectively; Waters Corporation, Milford, MA) and a reverse-phase ion exclusion column (Bio-Rad Aminex HPX-876H; Bio-Rad Laboratories, Hercules, CA). Measurements of ammonia-N were performed using a pH/ion selective electrode meter fitted with an ammonia-specific electrode that was equipped with a hydrophobic gas-permeable membrane. Fresh sample (5 g) was diluted in 100 mL of distilled water and mixed for 30 min using a magnetic stir plate. The probe was submerged into the solution and 1 mL of 10 *N* NaOH added and ammonia-N recorded.

Data were analyzed using Proc Mixed of SAS (SAS Institute Inc., Cary, NC) with the Fixed effect of ensiling time. Means were determined using the LSMEANS statement and were compared using the PDIFF option. Means with different superscript letter groups were obtained with PDMIX SAS macro (Saxton, 1998). Orthogonal contrasts were used to evaluate linear and quadratic responses to ensiling time. Because treatments were unequally spaced, contrast coefficients were determined using Proc IML of SAS (SAS Institute Inc.). Statistical significance and tendencies were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

Exp. 2

An unfermented HMC sample obtained at harvest from the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) in October 2013 was immediately frozen and stored at −20°C until March 2015. The sample was thawed in a refrigerator set for 3°C, homogenized, and divided into 33 subsamples of 250 g each using a quartering technique as described for Exp. 1. Three