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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

subsamples were randomly selected as fresh samples, and the remaining 30 subsamples were vacuum sealed in plastic bags and randomly assigned to 10 treatments (3 repetitions per treatment). Treatments were mini-silos fermenting in the dark either in a warm (at room temperature 20°C; **WR**) or cold (in the refrigerator set for 3°C; **CD**) temperature and allowed to ferment for 1, 3, 7, 14, or 28 d.

Fresh samples were analyzed for DM and mean particle size at the University of Wisconsin–Madison for material characterization (Table 1). Dry matter was determined in a forced-air oven set at 60°C for 48 h. Dried, unground samples were used for mean particle size determination using a Tyler Ro-Tap Shaker Model RX-29 (Mentor, OH) with sieves of 4,760-, 2,380-, 1,191-, 595-, 297-, 149-, and 63-μm apertures plus the bottom pan; mean particle size and surface area were calculated using a log normal distribution (Baker and Herrman, 2002). All ensiled samples were analyzed for DM, fermentation profile, and ammonia-N (% DM) at Rock River Laboratory Inc. (Watertown, WI) as described for Exp. 1.

Data were analyzed using Proc Mixed of SAS (SAS Institute Inc.) with the Fixed effects of temperature, ensiling time, and their interaction. 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). Statistical significance and tendencies were declared at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

Exp. 1—WPC

Effects of storage length on fermentation profile of WPC upon thawing are in Table 2. Content of DM did not differ ($P = 0.31$; 36.1% on average). Measurements of pH were affected by ensiling time ($P = 0.001$), with a quadratic decline observed after only 1 d (5.23 vs. 5.49–5.59) of fermentation and a gradual decrease until 28 d (3.84). Typical pH for well-fermented corn silage between 30 and 40% DM is 3.7 to 4.2 (Kung and Shaver, 2001). In addition, WPC of various maturities, hybrids, and processing ensiled in vacuum-sealed bags and allowed to ferment for 30 d averaged 3.72 in the studies by Ferraretto et al. (2015a,c). Gradual decrease in pH is related to the gradual increase ($P = 0.001$) in lactate and acetate concentrations from 1 d (0.88 and 0.39%, respectively) to 14 d of fermentation (5.48 and 1.22%, respectively). These values are within the typical concentration range reported by Kung and Shaver (2001) and are similar to values reported by Ferraretto et al. (2015a,c) after 30 d of fermentation. Total acid concentrations followed the same pattern ($P = 0.001$). Propionate concentration did not differ ($P = 0.77$; ranged 0.00 to 0.05% of DM), whereas butyrate was not detected. These data are in agreement with guidelines for corn silage reported by Kung and Shaver (2001). Concentration of succinate increased ($P = 0.001$) after 1 d of fermentation, peaked on 3 d, and decreased on 14 d. Ethanol concentration was greater ($P = 0.001$) for 2, 3, 7, 14, and 28 d (0.32% on average) compared with 0, 0.5, and 1 d (0.03% on average). Although these values were lower than the typical ethanol concentration reported by Kung and Shaver (2001), values were similar to WPC ensiled under similar conditions and allowed to ferment for 30 d (Ferraretto et al., 2015a,c). Ammonia-N increased ($P = 0.001$) 3-fold from 0 to 28 d of fermentation (0.02 vs. 0.06%, respectively). Overall, data from the current experiment suggest that fermentation occurred normally, and that upon thawing, the WPC maintained fermentation capacity.

Exp. 2—HMC

Effects of storage length and temperature on fermentation profile of HMC are in Table 3. Content of DM was greater for CD than WR ($P = 0.01$; 71.5 vs. 71.1%, on average), but this magnitude of difference is not likely of much biological consequence and may be related to fermentative losses volatilized during the drying process. A temperature × ensiling time interaction was observed ($P < 0.001$) for pH, ammonia-N, lactate, acetate, ethanol, and total acid concentrations. All parameters followed a similar pattern with gradual reduction in pH (6.27, 5.40, 4.97, 4.93, and 4.72, respectively), or increases in ammonia-N (0.00, 0.01, 0.01, 0.02, and 0.02% of DM, respectively), lactate (0.12, 0.24, 0.39, 0.45, and 0.43% of DM, respectively), acetate (0.00, 0.11, 0.15, 0.17, and 0.19% of DM, respectively), ethanol (0.00, 0.09, 0.17, 0.20, and 0.20% of DM), and total acid (0.12, 0.35, 0.54, 0.68, and 0.72% of DM, respectively) concentrations as fermentation progressed from 1 to 28 d in WR. In contrast, except for a slight difference in pH for 28 d compared with 1 d (6.41 vs. 6.48, respectively) of fermentation, ensiling time did not affect other fermentation parameters in CD. Values of pH, lactate, acetate, and ethanol concentrations in WR after 28 d of fermentation were 4.72, 0.43, 0.19, and 0.20%, respectively. Typical pH and lactic acid concentration for ensiled HMC between 70 and 75% DM content is 4.0 to 4.5 and 0.5 to 2.0, respectively (Kung and Shaver, 2001). However, when HMC was ensiled in vacuum-sealed bags under similar conditions to the present study and allowed to ferment for 30 d, pH was 4.57 and lactic acid concentration 0.39% (Ferraretto et al., 2015b). In addition, acetate and ethanol concentrations were within the ranges suggested by Kung and Shaver (2001) and similar to the results of Ferraretto et al. (2015b). These results suggest that HMC maintains fermentation capacity upon thawing. However, our data underscore that fermentation will be temperature dependent after thawing. A recent study by Zhou et al. (2016) reported lower pH and water-soluble carbohydrate concentrations for WPC fermented at 20°C compared with 5, 10, and 15°C after 60 d of ensiling. Furthermore, except for WPC ensiled at 5°C, adequate pH levels were reported for all treatments after 30 d of ensil-

Table 2. Effect of storage length on fermentation profile in thawed whole-plant corn

Item, % of DM unless noted otherwise	0 d	0.5 d	1 d	2 d	3 d	7 d	14 d	28 d	SEM	*P*-value[1]		
										Trt	L	Q
DM, % as fed	37.5	36.5	36.1	36.1	34.2	33.7	36.5	37.8	3.6	0.31	0.87	0.17
Ammonia-N	0.02^{e}	0.02^{e}	0.02^{de}	0.03^{cd}	0.04^{c}	0.05^{b}	0.06^{ab}	0.06^{a}	0.01	0.001	0.001	0.001
pH	5.49^{a}	5.59^{a}	5.23^{b}	4.47^{c}	4.22^{d}	3.98^{e}	3.88^{ef}	3.84^{f}	0.04	0.001	0.001	0.001
Lactic acid	0.24^{e}	0.26^{e}	0.88^{e}	2.14^{d}	3.00^{c}	4.43^{b}	5.48^{a}	4.71^{ab}	0.27	0.001	0.001	0.001
Acetic acid	0.00^{f}	0.12^{f}	0.39^{e}	0.71^{d}	0.93^{c}	0.96^{c}	1.22^{b}	1.58^{a}	0.05	0.001	0.001	0.001
Propionic acid	0.00	0.00	0.04	0.04	0.00	0.05	0.04	0.00	0.04	0.77	0.37	0.57
Ethanol	0.00^{b}	0.00^{b}	0.09^{b}	0.26^{a}	0.35^{a}	0.31^{a}	0.34^{a}	0.36^{a}	0.05	0.001	0.001	0.001
Total acid	0.24^{f}	0.37^{f}	1.56^{e}	3.15^{d}	4.25^{c}	5.74^{b}	6.94^{a}	6.54^{ab}	0.32	0.001	0.001	0.001

$^{a–f}$Means in the same row with different superscripts differ ($P \leq 0.05$).

[1]Trt = treatment effect, L = linear effect, and Q = quadratic effect.

Table 3. Effect of storage length and temperature on fermentation profile in thawed high-moisture corn

Item, % of DM unless noted otherwise	Cold[1]					Warm[1]						*P*-value[2]		
	1 d	3 d	7 d	14 d	28 d	1 d	3 d	7 d	14 d	28 d	SEM	Temp	Time	Int
DM, % as fed	71.6	71.2	71.7	71.9	71.3	71.4	71.1	70.7	71.1	71.2	0.2	0.01	0.49	0.19
Ammonia-N	0.00^{e}	0.00^{de}	0.00^{d}	0.01^{c}	0.01^{c}	0.00^{d}	0.01^{d}	0.01^{c}	0.02^{b}	0.02^{a}	0.001	0.001	0.001	0.001
pH	6.48^{a}	6.47^{ab}	6.44^{ab}	6.45^{ab}	6.41^{b}	6.27^{c}	5.40^{d}	4.97^{e}	4.93^{e}	4.72^{f}	0.02	0.001	0.001	0.001
Lactic acid	0.10^{d}	0.10^{d}	0.10^{d}	0.11^{d}	0.14^{d}	0.12^{d}	0.24^{c}	0.39^{b}	0.45^{a}	0.43^{ab}	0.02	0.001	0.001	0.001
Acetic acid	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.11^{c}	0.15^{b}	0.17^{b}	0.19^{a}	0.01	0.001	0.001	0.001
Ethanol	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}	0.09^{b}	0.17^{a}	0.20^{a}	0.20^{a}	0.02	0.001	0.001	0.001
Total acid	0.10^{d}	0.10^{d}	0.10^{d}	0.11^{d}	0.14^{d}	0.12^{d}	0.35^{c}	0.54^{b}	0.68^{a}	0.72^{a}	0.03	0.001	0.001	0.001

$^{a–f}$Means in the same row with different superscripts differ ($P \leq 0.05$).

[1]Cold = samples fermented in the refrigerator set for 3°C, Warm = samples fermented at room temperature 20°C.

[3]Temp = temperature effect, Time = ensiling time effect, and Int = interaction.

ing (Zhou et al., 2016). Wang and Nishino (2013) ensiled TMR at 5, 15, 25, and 35°C for up to 90 d. The authors reported lower lactic acid bacteria count, lactic and acetic acid concentrations, and corresponding higher pH and yeast count for silage ensiled at 5°C. These results support our premise that colder temperatures inhibit fermentation in silage. Perhaps CD temperature in our study was too low to allow for initial fermentation in HMC, and future research is warranted to elucidate the temperature threshold at which fermentation starts under cold weather. Zhou et al. (2016) also evaluated the effect of temperature during fermentation on bacteria diversity in WPC. The authors concluded that low temperatures change patterns of the lactic acid bacteria population responsible for silage fermentation. Thus, future research is warranted to elucidate potential microbial inoculants to improve fermentation under low temperature.

CONCLUSIONS

Fermentation capacity in WPC upon thawing was maintained even after WPC was frozen for a prolonged period in storage. In addition, although HMC maintains fermentation capacity upon thawing even after being frozen for a prolonged period in storage, fermentation will not occur until a warm temperature is reached. Further research is warranted to elucidate at which temperature fermentation progresses normally after thawing.

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