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Effects of Water-Soluble Carbohydrate Content on Silage Fermentation of Wheat Straw

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To determine a suitable initial water-soluble carbohydrate (WSC) content to make wheat straw natural fermentation successful and to study fermentation characteristics, glucose was used to adjust the initial WSC content to 1.4%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, and 10.0% dry matter (DM) in the wheat straw. At 30 d of fermentation, there were three pHs: when the initial WSC content was 1.4%, the pH was 5.5; when the initial WSC contents were 4.0%, 5.0% and 6.0%, the pHs were near 5.1; and when the initial WSC contents were 7.0%, 8.0%, 9.0% and 10.0%, the pHs were near 4.0. The pattern of changes in WSC content during the fermentation was similar to that in pH. At 30 d of fermentation, there existed a dividing line in WSC remnants between the initial 6.0% WSC treatment and the initial 7.0% WSC treatment. When the initial WSC content was more than 7.0%, the remaining WSC content was more than 23.7 g/kg DM. When the initial WSC content was less than 6.0%, the remaining WSC content was less than 13.6 g/kg DM. Particularly for the 1.4% WSC treatment, the remaining WSC content was 2.1 g/kg DM. The results of the microbiological enumeration showed that with the increase in initial WSC content, the numbers of lactic acid bacteria (LAB) and other bacteria generally decreased. Denaturing gradient gel electrophoresis (DGGE) results showed that when the initial WSC content was beyond 7.0%, the LAB of the fermentation system were detected.

[**Key words:** wheat straw, silage, fermentation, water-soluble carbohydrates, denaturing gradient gel electrophoresis]

Wheat straw, an agricultural residue, is abundantly generated annually around the world. In northern America and Europe, nearly 300 million tons of wheat straw are produced annually. The average yield of wheat straw is 1.3–1.4 kg/kg grain, which results in a considerable amount of surplus straw (1). In China, the annual yield of main crop straw and stalk is approximately 604 million tons, including 115.4 and 139.6 million tons of wheat and rice straws, respectively. Approximately 50% of these biomass sources are burned for cooking and heating or left or combusted directly in fields, such that considerable crop straw and stalk resources are wasted and serious environmental pollution occurs (2). Although tremendous efforts have been made to convert these biomass sources into value-added products, they have not yet been fully utilized (3). During winter in northern parts of China, the amount of feed of ruminants is inadequate. If these biomass sources can be effectively converted into fodder, it will have a tremendous impact on the development of stockbreeding in China.

Ensiling is a forage crop preservation method that has been utilized for centuries. A combination of anaerobic condition and acidity protects the forage from the proliferation of deleterious bacteria and fungi, and it also increases the palatability of the forage due to lactic acid production (4–6). To obtain a high-quality fermented product, many additives have been used during silage fermentation (7). Seale *et al*. found that sugar is a limiting factor in producing good-quality fermented products (8). Sugar mainly serves as a carbon source for microorganisms. Molasses, lactose and a mixture of cereal grains and malt, dextrose, corn or tapioca flour have been used as additives (9).

Straw is mainly composed of cellulose, hemicellulose and lignin, hence its low energy, low digestibility and low protein content prevent its use in feedlots. It is directly ensiled; however, it produces a silage quality due to its low water-soluble carbohydrate (WSC) content (4). The addition of sugar is a good way of improving the silage quality. In previous reports (8, 10–12), researchers focused on how much sugar has been added and what addition level is suitable for successful fermentation. Studies of the effects of WSC content in materials used for ensiling have seldom been reported, particularly in crop straws.

In this study, we fermented wheat straw with different WSC contents to determine the optimal WSC content for fermentation. Furthermore, we studied the fermentation char-

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acteristics of the obtained products.

MATERIALS AND METHODS

Materials Wheat (*Triticum aestivum*) straw was obtained from the experimental farm of China Agricultural University, Beijing, China. After harvesting, the wheat straw was air-dried. It had the following composition: dry matter (DM), 900 g/kg raw materials; crude protein, 42 g/kg DM; WSC, 14 g/kg DM; ash, 96.3 g/kg DM, and crude fiber, 359 g/kg DM. It was chopped to approximately 1 cm long, and stored for fermentation. Glucose was bought from Beijing Chemical Reagent Company, China.

Ensiling Glucose was used to adjust WSC content in the wheat straw to 1.4%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, and 10.0% DM before fermentation. Deionized water was sprayed to the wheat straw and the final moisture was approximately 70% of the mixtures. The mixtures were ensiled in 250-ml test-tube silos and were then fermented under anaerobic condition at 30°C. There were 15 silos for each treatment. Three silos were opened on days 2, 6, 10, 15 and 30 after ensiling for chemical and microbiological analyses.

Chemical analysis pH was determined using the compact pH meter (model B-212; Horiba, Kyoto). DM was determined by oven drying for 48 h at 60°C. Ash content was obtained after 3 h at 550°C (6). Crude protein and crude fiber contents were determined according to Association of Official Agricultural Chemists (AOAC) procedures (13). The WSC contents of samples were determined by colorimetry after reaction with an anthrone reagent (14). A fermentation product of 1.5 g fresh matter (FM) of fermentation was placed in 10-ml test tube containing 3 ml of sterile water for 30 min to filter the juice for the detection of organic acids, ethanol and glycerol by gas chromatography. The following procedures were applied in the gas chromatography (model GC-17A; Shimadzu, Kyoto): 1 μl of filtered juice was injected into the capillary column (CP-Chirasil-Dex CB, 25 m × 0.25 mm); the split ratio was 13 : 1; the injector temperature was 190°C; the detector temperature was 200°C; and the column temperature program was set up as follows: initial 50°C (hold 1 min), a rate of 18°C per min up to 190°C, and final 190°C (hold 3 min). The column head pressure was 91 kPa. The column flow rate was 5.0 ml min^{-1}. Data were analyzed by the Shimadzu Class-GC10 data processing system. Each analysis was performed in triplicate.

Data were subjected to ANOVA using the general linear model procedure of the Statistic Analysis System (ver. 6.12; SAS Inst., Cary, NC, USA).

Microbiological examination At 30 d of fermentation, lactic acid bacteria (LAB) and other bacteria were enumerated. LAB were enumerated via the pour-plate technique in MRS agar (15). Other bacteria were cultured in nutrient agar (16). Plates were incubated at 30°C for 48 h. Nine plates were counted for each treatment.

DGGE analysis of microbial communities in fermented product To analyze the complexity of the microbial communities during fermentation, the DNA of the fermented product for each treatment was extracted by the benzyl chloride method (17). The extracted DNA was used as the template in PCR amplification. The primers used for amplifying the V3 region of 16S rDNA were the following: 357F-GC, 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′ (*Escherichia coli* positions, 341 to 357), and 517R, 5′-ATTACCGCGGCTGCTGG-3′ (*E. coli* positions, 517 to 534). The underlined sequence is a GC clamp (18, 19). Initial DNA denaturation was performed at 95°C for 10 min, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and elongation at 72°C for 1 min 10 s, and then a final elongation step at 72°C for 5 min. Denaturing gradient gel electrophoresis (DGGE) was performed according to the method described by Muyzer *et al.* (18). The V3 region bands of 16S rDNA on the gel were stained with SYBR Green I (20). The bands on DGGE gels were excised and the DNA was recovered and reamplified with the primers 357F (5′-CCTACGGGAGGCAGCAG-3′) and 517R (5′-ATTACCGCGGCTGCTGG-3′) (19). Initial DNA denaturation was performed at 95°C for 10 min, followed by 25 cycles of denaturation at 93°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 1 min 30 s, and then a final elongation step at 72°C for 5 min. The amplified fragments were purified using the high pure PCR product purification kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The purified PCR fragments were sequenced using the ABI 3730XL DNA sequencer (Perkin Elmer China) at SunBiotech Developing Center (Beijing, China).

The sequence similarity searches were performed in the GenBank data library using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). The V3 region sequences of 16S rDNA were compared with sequences from strains registered in the GenBank. The sequence information was then imported into the CLUSTAL X software program for assembly and alignment (21). A phylogenetic tree was constructed by the neighbor-joining method (22). The V3 region sequences of 16S rDNA generated in this study were deposited in the GenBank data library under accession nos. DQ123808 to DQ123816.

RESULTS AND DISCUSSION

Changes in pH during fermentation pHs decreased in all treatments at the beginning of fermentation (Fig. 1). However, the pHs in the 1.4% and 4.0% WSC treatments decreased more gradually than those in the 5.0–10.0% WSC treatments at 2 d of fermentation. As fermentation progressed, pH changes showed different patterns. At 30 d of fermentation, there were three pHs: when the initial WSC content was 1.4%, the pH was 5.5; when the initial WSC contents were 4.0%, 5.0% and 6.0%, the pHs were near 5.1;

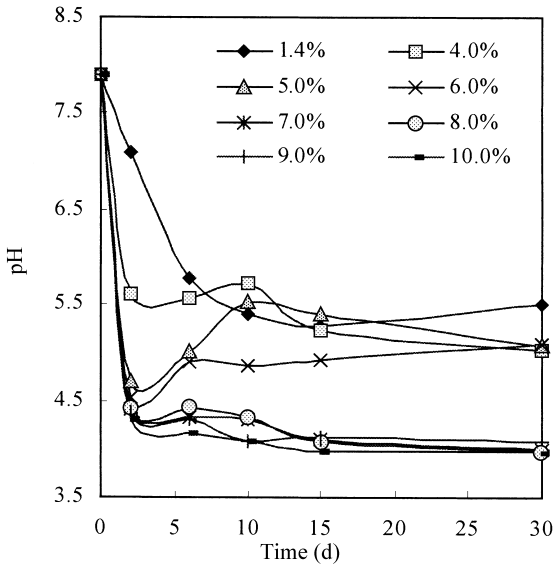

FIG. 1. Changes in pH during fermentation. The treatments of initial WSC contents before fermentation were 1.4%, 4.0%, 5.0%, 6.0%, 7.0%, 8.0%, 9.0%, and 10.0%.