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Changes in Mono–and Disaccharides Compositions of Guineagrass (*Panicum maximum* Jacq.) Silage During Early Stages of Ensiling

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The dynamics of fermentation during the early stage of ensiling was studied with guineagrass (*Panicum maximum* Jacq.) ensiled in the laboratory silos of 100 ml capacity. The silos were kept in the room set at 25 °C, and then were opened on 0.5, 1, 2, 3, 5 and 7 days after ensiling, respectively. The samples were taken from three silos at each sampling time for chemical analyses. The results showed a slow decrease in pH and a slow increase in lactic acid content during the early stage of ensiling. The rate and extent of the full fermentation process was restricted, causing a high final pH value, low contents of lactic acid, acetic acid, total VFAs and total organic acids. No or only small amounts of butyric acid, valeric acid and propionic acid and low values of AN/TN at the end of ensiling implied that the silage was stable. However, there was an evidence that ethanol increased continuously as the major fermentation product and only detected level of residual mono–and disaccharides (fructose, glucose and sucrose) were found at the end of the experiment, indicating that alcoholic fermentation had taken place due to yeasts activity. The contents of mono–and disaccharides showed the largest decreases within the initial 0.5 day of ensiling, however, very low contents of fermentation products were detected, suggesting that the loss was caused mainly by plant respiration in this period. The rate of reduction in mono–and disaccharides compositions within initial 5 days of ensiling was ranked in the order of glucose>fructose>sucrose, suggesting that glucose and fructose might be more favorably utilized by microorganisms than sucrose.

INTRODUCTION

It is well known that air is still present in the silage during the early stage of ensiling and this enables plant respiration and aerobic microbial activity to take place, causing a loss of both fermentable substrates and nutritive materials. The rate and efficiency of acid production in the first stage of fermentation are important factors to both silage making and quality (Weinberg *et al.*, 1988), therefore, the initial fermentation characteristics are critical to the success or failure of silage making.

Tropical grasses are generally low in water soluble carbohydrate (WSC) but high in polysaccharide contents (Smith, 1962) and have coarse, porosity and stemmy structures

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(Catchpoole and Henzell, 1971). Therefore, they are usually less dense and presumably more permeable, and relativity large quantities of air may be trapped in the forage mass than its temperate counterparts just after ensiling. These could cause a difference in the ensiling process and the silage quality between these species types (Kim and Uchida, 1990; Catchpoole and Henzell, 1971). According to McDonald *et al.* (1991), in temperate origin fructans are the most abundant WSC, however, grasses of tropical and subtropical originals accumulate starches instead of fructans. Yokota *et al.* (1991) and Miyagi *et al.* (1993) reported that a main preservative acid in napiergrass silage was lactic acid (LA). However, some researchers (Kim and Uchida, 1990; Catchpoole and Henzell, 1971) described that a main preservative acid in silages made from tropical grasses was acetic acid (AA). The WSC content is generally lower in tropical grasses than in temperate grasses, and how AA dominance proceeds during the fermentation of tropical grass silage is still not clear. Silages made from a number of tropical grasses have proved stable against anaerobic decomposition in the silo, as judged by low ratio of ammonia nitrogen to total nitrogen (AN/TN) and low butyric acid (BA) content, but their chemical composition does not conform to temperate standards for LA silage (Catchpoole and Henzell, 1971).

Guineagrass (*Panicum maximum* Jacq.) is one of the most important tropical forage crops. It is now widely distributed through tropical and subtropical regions of the world, and is often used as a silage making material. However, there is limited information regarding the changes in mono–and disaccharides and the production of organic acids during the early stage of ensiling. The purpose of the present work is to study the relation between the changes in mono–and disaccharides compositions and the fermentation quality in the early stages of guineagrass silage.

MATERIALS AND METHODS

Silage making

Guineagrass was cultivated in the experimental field of Kyushu University, Hakozaki, Fukuoka, Japan. The second growth of guineagrass was harvested at the milky ripe stage using a hand sickle on 18 October 1999. The harvested guineagrass was chopped into approximately 1 cm length with a forage cutter. The chopped grass was immediately collected and 85 grams were packed into a plastic laboratory silo (100 ml capacity) in triplicates, followed by being sealed with a screw top and stored in the room kept at 25 °C. The silos were opened on 0.5, 1, 2, 3, 5 and 7 days after ensiling.

Chemical analyses

The chopped grass was immediately collected for determining the contents of dry matter (DM), TN, crude protein (CP) and mono–and disaccharides compositions (fructose, glucose and sucrose). After the silos were opened and the contents were mixed thoroughly, 30 grams of the sample were taken from each silo. This was followed by adding 60 grams of distilled water and extracting at 4 °C for 24 hours. Then, the extracts were filtered through two layers of cheesecloth and a filter paper (Toyo No. 5A) and the filtrates were stored at −20 °C prior to chemical analyses. The filtrates were used for determining pH, AN, LA, ethanol, and volatile fatty acids (VFAs). The pH of silage was measured using a glass electrode pH meter (Horiba Co, Japan). TN was analyzed by the

Kjeldahl method (AOAC, 1984) and AN with an ammonia electrode (Model IM-22P, Toa Electronics Ltd, Japan). CP was determined with 6.25 multiplied by TN. The LA content was determined using the method of Barker and Summerson (1941), and VFAs and ethanol with gas chromatography (Shimadzu GC-17A, Japan, with 12 m capillary column, condition: column temperature 100 °C, injection temperature 250 °C). The DM contents of the grass and silages were determined by drying in an oven at 65 °C for at least 48 hours (AOAC, 1984), and DM of silage was recalculated with the contents of volatile compositions. Mono-and disaccharides compositions of the fresh guineagrass and silages were determined by high performance liquid chromatography (HPLC) as shown in our previous report (Shao *et al.*, 2002).

Statistical analyses

All data were analyzed statistically by one-way analysis of variance with storage periods as a factor and statistical significance among storage periods for each item was determined by Fisher's least significant difference test; these were performed by ANOVA using the GLM procedure of the Statistical Analysis System (SAS, 1984).

RESULTS

The characteristics of guineagrass before ensiled were as follows; it contained intermediate contents of DM (258.64 g/kg) and CP (79.35 g/kg), higher contents of fructose (33.54 g/kg), glucose (30.54 g/kg), sucrose (80.27 g/kg) and total mono-and disaccharides (144.34 g/kg).

The early fermentation characteristics of the guineagrass silage are presented in Table 1. The pH value decreased slowly from 5.68 to 5.35, a slight but significant ($p < 0.05$) decrease from 2 days of ensiling. The LA content increased slowly from 1.53 to 6.15 g/kg, a small but significant ($p < 0.05$) increase from 2 days of ensiling. The AA

Table 1. Changes in fermentation quality of guineagrass during the early stage of ensiling

Item	Storage periods (days)					
	0.5 day	1 day	2 days	3 days	5 days	7 days
pH (SD)	5.68 (0.05) c[1)]	5.68 (0.02) c	5.56 (0.02) b	5.55 (0.05) b	5.56 (0.10) b	5.35 (0.10) a
DM (SD) (g kg^{-1})	256.10 (0.74) a	254.50 (4.22) a	253.89 (1.65) a	256.38 (3.25) a	254.64 (6.20) a	256.60 (7.05) a
Ethanol (SD) (g kg^{-1} DM)	2.49 (0.55) a	3.52 (0.57) a	5.36 (1.08) ab	7.83 (1.75) b	15.30 (4.84) c	20.76 (0.88) d
LA (SD) (g kg^{-1} DM)	1.53 (0.21) a	2.23 (0.68) ab	3.85 (0.20) bc	5.48 (0.82) cd	5.01 (2.13) cd	6.15 (0.69) d
AA (SD) (g kg^{-1} DM)	0.55 (0.10) a	1.03 (0.11) a	1.04 (0.14) a	0.93 (0.04) a	3.80 (0.53) b	4.42 (0.61) c
BA (SD) (g kg^{-1} DM)	0.14 (0.17) a	0.02 (0.04) a	0.00 (0.00) a	0.07 (0.00) a	0.39 (0.27) a	2.71 (1.30) b
VA (SD) (g kg^{-1} DM)	0.13 (0.13) b	0.00 (0.00) a	0.00 (0.00) a	0.00 (0.00) a	0.02 (0.04) a	0.02 (0.04) a
PA (SD) (g kg^{-1} DM)	0.32 (0.21) a	0.09 (0.15) a	0.00 (0.00) a	0.00 (0.00) a	0.06 (0.05) a	0.91 (0.48) b
VFAs (SD) (g kg^{-1} DM)	1.14 (0.55) a	1.13 (0.29) a	1.04 (0.14) a	1.01 (0.03) a	4.28 (0.45) b	8.12 (2.12) c
Organic acids (SD) (g kg^{-1} DM)	2.66 (0.49) a	3.37 (0.96) ab	4.89 (0.20) bc	6.48 (0.83) c	9.29 (1.72) d	14.22 (1.82) e
LA/AA (SD)	2.83 (0.71) bc	2.14 (0.43) ab	3.76 (0.56) c	5.86 (0.93) d	1.39 (0.79) a	1.42 (0.33) a
AN (SD) (g kg^{-1} TN)	2.64 (0.04) a	9.01 (2.26) ab	15.81 (1.29) bc	21.19 (1.23) c	35.55 (3.66) d	45.54 (9.22) e

1) Values followed by different letters in the same row show significant differences at $p < 0.05$.

Table 2. Changes in contents of mono–and disaccharides of guineagrass during the early stage of ensiling (g kg^{-1} DM)

Item	Storage periods (days)						
	0 day	0.5 day	1 day	2 days	3 days	5 days	7 days
Fructose (SD)	33.54	21.39 (3.77) cd[1)]	24.42 (2.18) d	15.26 (6.23) bc	9.63 (3.81) b	1.62 (1.57) a	0.87 (0.75) a
Glucose (SD)	30.54	17.65 (4.79) c	14.48 (1.51) c	6.13 (5.44) b	1.93 (3.35) ab	0.00 (0.00) a	0.00 (0.00) a
Sucrose (SD)	80.27	34.22 (8.90) c	34.32 (7.34) c	16.48 (5.01) b	6.08 (2.15) ab	8.85 (8.77) ab	0.00 (0.00) a
Mono–and disaccharides (SD)	144.34	73.26 (8.34) d	73.22 (8.02) d	37.86 (10.07) c	17.64 (7.78) b	10.47 (10.32) ab	0.87 (0.75) a

1) Values followed by different letters in the same row show significant differences at $p<0.05$.

content did not greatly change within initial 3 days of ensiling, and then increased significantly ($p<0.05$) to 4.42 g/kg. LA/AA gradually increased and reached the peak (5.86) on the day 3 of ensiling ($p<0.05$), and then decreased significantly ($p<0.05$) to 1.42. The total VFAs content showed a similar profile with AA, almost constant within the initial 3 days, and then increased significantly ($p<0.05$) to 8.12 g/kg at the end of the experiment. Total organic acids increased significantly ($p<0.05$) from 2 days of ensiling, and reached 14.22 g/kg at the end of ensilage. The ethanol content gradually increased within initial 2 days of ensiling and then kept on increasing ($p<0.05$) to 20.76 g/kg on the 7 th day. The contents of BA, valeric acid (VA) and propionic acid (PA) were hardly detected during the initial 5 days of ensilage, however, there was a small but significant ($p<0.05$) increase in BA and PA contents on the 7th day of ensiling. AN/TN significantly ($p<0.05$) increased from 2 days of ensiling and reached 45.54 g/kg at the end of ensiling. DM content did not change greatly up to 7 days of ensiling.

Changes in the contents of mono–and disaccharides during the ensiling are shown in Table 2. There were the largest decreases in mono–and disaccharides compositions (fructose, glucose and sucrose) within initial 0.5 day of ensiling from those of the original fresh guineagrass. Fructose tended to decrease ($p>0.05$) between 0.5 and 2 days, and then decreased significantly ($p<0.05$) from 3 days to 0.87 g/kg at the end of ensilage. Glucose decreased slowly between 0.5 day and 1 day of ensiling, and then decreased significantly ($p<0.05$) to zero until 5 days of ensilage. Sucrose showed no changes between 0.5 day and 1 day of ensiling, and then decreased significantly ($p<0.05$) to zero on the 7th day of ensiling. The rate of disappearance in mono–and disaccharides compositions was ranked in the following order: glucose > fructose > sucrose within initial 5 days of ensiling.

DISCUSSION

Evidence has shown that the rate and extent of guineagrass silage fermentation was restricted throughout the ensiling period in this experiment. This was well indicated by a slow decrease in pH and a slow increase in LA content, resulting in high pH value, low contents of LA, AA, VFAs and total organic acids, and low AN/TN value at the end of ensiling. These results were different from our previous study (Shao *et al.*, 2002), however, seem to be similar to the fermentation quality of wilted silages (McDonald *et al.*, 1968; Anderson and Jackson, 1970; Marsh, 1979; Driehuis *et al.*, 1997). We harvested guineagrass at the milky ripe stage with intermediate DM content (258.64 g/kg) and high

mono–and disaccharides content (144.34 g/kg) but the material plant was very rigid. Nevertheless, the fermentation process was restricted. This could probably be attributed to the rigid physical properties of guineagrass at the milky ripe stage. It had a coarse, porosity and stemmy structures, and thus there might be a relatively larger quantity of air trapped in the forage mass just after ensiling, causing the delay in time for the air disappearance. These factors would make the cell breakdown and release of plant juice more difficult and slower, thereafter restricted the rate and extent of fermentation of guineagrass silage by epiphytic LAB. Greenhill (1964a, b, c) also reported that cell breakdown and release of intra–cellular plant juices are prerequisite for the initiation of LAB fermentation, and the complete exclusion of fresh air from the silage mass can usually be expected to result in cell breakdown and juice release.

The ethanol content continuously increased as a major fermentation product whereas mono–and disaccharides compositions (fructose, glucose and sucrose) continuously decreased throughout the fermentation process. There was a larger amount of ethanol compared to LA and AA, and it was likely that yeasts (aerobic bacteria) were responsible for the observed ethanol production (McDonald *et al.*, 1991). The reasons for the predominance of ethanol rather than LA fermentation when the guineagrass high in fermentable sugers were ensiled in anaerobic conditions are not clear. McDonald *et al.* (1968) found more yeasts in silages of high DM content. In the present study it was probably due to the essentially different physical structure of guineagrass at the milky ripe stage as compared with temperate grasses, resulting in the slow release of juice and inhibition of the LA fermentation. The high pH value and low level production of LA and AA might stimulate yeast activity during ensiling (Alli *et al.*, 1985; Driehuis *et al.*, 1997). In addition there was a small but significant high BA and PA contents on the 7 th day of ensiling, which indicated some clostridial fermentation occurring. This was attributed to the high pH and some residual sucrose on the 5 th day of ensiling. Although there were a high pH value and low LA content at the end of ensiling, low AN/TN value (45.54 g/kg) and no or only small amounts of BA, VA and PA detected during ensilage indicated a stable silage (Catchpoole and Henzell, 1971). Yokota *et al.* (1991) and Miyagi *et al.* (1993) reported that the ensiling nature of tropical species was LA fermentation, but others (Catchpoole and Henzell, 1971; Kim and Uchida, 1990) demonstrated that a main preservation in silages made from tropical grasses was AA. These were different from our present results, in which guineagrass had high mono–and disaccharides content but did not show LA or AA fermentation. The ethanol was a major fermentation product and almost all of mono–and disaccharides disappeared at the end of the experiment. This suggests that the physical structure of tropical grasses is also one of the important factors affecting the fermentation quality in the experiment. DM content did not change greatly up to 7 days of ensiling, which was due to the property of laboratory silo that was efficiently sealed and no seepage loss.

The contents of mono–and disaccharides compositions (fructose, glucose and sucrose) showed the largest decreases within initial 0.5 day of ensiling (Table 2), and very small amounts of fermentation products were detected (Table 1), suggesting that the loss was caused mainly by plant respiration in this period. This is similar to that found by other workers (Wylam, 1953; Carpintero *et al.*, 1969; Seale, 1986). The rate of reduction in mono–and disaccharides compositions within initial 5 days of ensiling was ranked in the

order of glucose>fructose>sucrose, suggesting that glucose and fructose might be more favorably utilized by microorganisms than sucrose. There were no initial rises in fructose and glucose content, which is different from previous studies on temperate grass silages (Masaki and Ohyama, 1979, Shao *et al.*, 2002). This may be due to the absence of fructans in guineagrass, because tropical grasses accumulate starches instead of fructans.

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