# FERMENTATION QUALITY EVALUATION OF ITALIAN RYEGRASS SILAGES BY USING THE BUFFER INDEX CURVE

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## Summary

This study was conducted to demonstrate the practical use of the buffer index curve as a criterion of silage quality evaluation. Forty five samples of the ensiled Italian ryegrass were collected from farms in Okayama, Japan.

Silages were devided into 4 groups by the pattern of buffer index curve. Heavy wilting silages (A group) showed no peak on buffer index curve and Flieg's score of 95.6. However, moderate or weak wilting silages (B group) with moisture content of 63.3 showed a peak at pH 4.0 and a Flieg's score of 67.8. Both of A and B groups were regarded as a good quality silage. Silages (C group) with a peak at pH 4.5 were of a medium quality with Flieg's score of 45.3. Poorly preserved silages (D group) with Flieg's score of 12.0 had a peak at pH 5.0.

There was a high positive correlationship ($p < 0.01$) between the peak value of buffer index curve and the organic acid content.

(**Key Words :** Acetic Acid, Buffer Index Curve, Butyric Acid, Flieg's Score, Lactic Acid, Silage Quality Evaluation)

## Introduction

In Europe and Asia, the assessment of silage quality has been based on an organoleptic evaluation, a distillation method of Flieg (1938) and a measurement of pH. Currently many laboratories employ gas chromatography (GC) to separate and determine individual volatile fatty acid, quantatively. However, there are drawbacks since these conventional methods are laborious and expensive, and may be subjective to misinterpretation. Particularly pH is unable to be used as the sole criterion for assessing silage fermentation because the stable condition of silage can be achieved at much higher pH with the dry matter level increased (Haigh, 1987; Uchida et al., 1989).

Kim and Uchida (1991) proposed a rapid, economical and reliable titration method that had advantages over the distillation method of Flieg and the chemical analysis by GC. By using 13 Italian ryegrass silage made in 3 *l* small silo, they found two patterns of buffer index curve and noticed that well preserved silages had a peak of buffer index curve at pH 4.0, regardless of wilting, whereas with poorly preserved silages had a peak at pH 5.0.

The objective of this study was to demonstrate that the buffer index curve could be used practically as a criterion of silage fermentation quality evaluation.

## Materials and Methods

Forty five samples of the ensiled Italian ryegrass (*Lolium multiflorum* Lam.) were collected from forty five farms in Okayama, Japan. Fresh silage (20 g) was suspended in 300 ml distilled water and stirred with a magnetic bar for 10 minute. The water extract was titrated to pH 3.0 with 0.1N HCl and then titrated to pH 10 with 0.1N NaOH. Initial pH and all further measurements taken during titration were recorded after 3 minute. The buffer index curve was derived from the titration curve by point-to-point calculation, as the buffer index (or buffer capacity, $\beta$) value equals the slope of the titration curve ($\Delta$NaOH/$\Delta$pH). That is, in this experiment, the

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Received May 13, 1992
Accepted August 18, 1992

buffer index value was determined from the amount of 0.1N NaOH consumed in titration of each 0.5 pH step.

Silage fermentation quality was determined by conventional chemical assessments such as Flieg method (1938), the composition of volatile fatty acid by gas chromatography (Uchida and Hayashi, 1985), lactic acid content by the method of Barker and Summerson (1941) and pH value by placing a 10 g sample in 100 ml distilled water. Moisture content of silage was determined by the freeze and vaccum dry method (Uchida, 1986).

## Results and Discussion

Buffer index curve were devided into four groups (figure 1). The silages of A Group (n = 10) had no peak on the buffer index curve. However, the peak position of B (n = 16), C (n = 15), or D (n = 4) group was around pH 4.0, 4.5 or 5.0, respectively. The most important organic acids in ensiled products are lactic, acetic and butyric acids, which have negative logarithm of dissociation constants (pKa) of 3.86, 4.76 and 4.82, respectively. The pKa values of these acids are numerically equal to the pH's at the peaks of buffer index curve (Perrin and Dempsey, 1973). Kim and Uchida (1991) reported that silage extracts produced buffer index curves with single peaks in the pH range of 3.5 to 5.5 and its position was depended on relative strengths of the acidic groups. They were also observed, by using the silages making in laboratory small silo, that lactate silage had a peak of buffer index curve at 4.0 and butyrate silage had a peak at pH 5.0. In this experiments, however, A and C groups were new finding beyond silage groups made in experimental small silo.

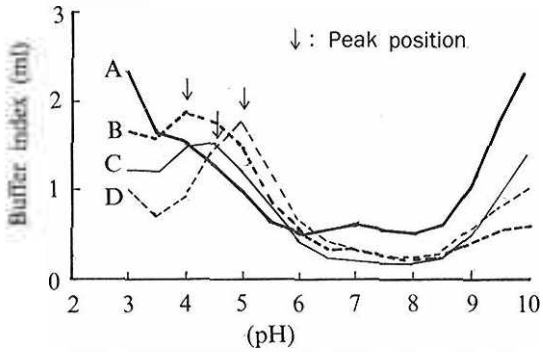


Figure 1. Typical buffer index curve of each silage group

The Flieg's scores of A, B, C and D groups were 95.6, 67.8, 45.3 and 12.0, respectively (table 1). For silages in A group, however, the moisture content was 46.2% and the pH was 5.49. From these data and low concentration of total acids (1.99%), it is noticed that A group was heavily wilted prior to ensiling. Wilting of the herbages prior to ensiling is regarded as a desirable process due to the inhibited clotridial activity as well as lactic acid bacterial activity, and the stable condition of the ensiled product can be achieved at much higher pH (Marsh, 1979). These facts account for why there were no peak on the buffer index curve in silages of A group. Therefore, in the silage assessment by buffer index curve, it will be able to regard silages with no peak as wilted ones.

The B group has undergone the extensive

TABLE 1. CHEMICAL COMPOSITIONS AND FLIEG'S SCORE OF SILAGE GROUPS

| Position of peak (pH) | Group of silage | | | |
|---|---|---|---|---|
| | A (no) | B (4.0) | C (4.5) | D (5.0) |
| | n = 10 | n = 16 | n = 15 | n = 4 |
| Moisture content (%) | 46.15 ± 12.45* | 63.26 ± 7.77 | 73.65 ± 5.71 | 72.46 ± 2.43 |
| pH | 5.49 ± 0.58 | 4.40 ± 0.21 | 4.42 ± 0.24 | 5.01 ± 0.17 |
| Lactic acid (% DM) | 1.40 ± 1.09 | 4.99 ± 1.64 | 4.83 ± 1.53 | 0.87 + 1.19 |
| Acetic acid (% DM) | 0.31 ± 0.25 | 1.08 ± 0.58 | 1.63 ± 1.22 | 1.01 ± 0.38 |
| Butyric acid (% DM) | 0.28 ± 0.42 | 1.02 ± 0.63 | 1.99 ± 1.05 | 4.13 ± 1.55 |
| Flieg's score (% DM) | 95.60 ± 6.70 | 67.75 ± 18.74 | 45.27 ± 22.58 | 12.00 ± 10.49 |

* Mean ± SD.

fermentation. Lactic acid production was vigorous and the pH value remained low. However, the silages in C group showed the conversion of lactic acid to butyric acid and some increase in pH. This group was of intermediate quality between the B group of lactate silage and the D group of butyrate silage in considering the peak position at pH 4.5 and the chemical assessment. The D group was badly spoiled. With the considerable conversion of lactic acid to butyric acid, the pH value also rose correspondingly.

Table 2 shows the correlation coefficients between the concentrations of acids and the peak values of buffer index curve. Of the three acids produced during the fermentation, only percentages of acetic acid in A and B groups was positively correlated with the peak value. The correlation coefficients of the ensiled C group indicated that buffer substance of lactic acid was gradually substituted by volatile fatty acids such as acetic and butyric acids. The peak values at pH 5.0 for D group were highly correlated ($r = 0.990$, $p < 0.01$) with the concentrations of butyric acid. These findings demonstrated that the peak positions of buffer index curve were decided by the main end products depending upon the type of silage fermentation.

Woolford (1984) has presented the schemes

TABLE 2. CORRELATION COEFFICIENTS BETWEEN THE CONCENTRATION OF ORGANIC ACIDS AND THE PEAK VALUE OF BUFFER INDEX CURVE

| | Peak value[a] | | | |
|---|---|---|---|---|
| | A (n = 10) | B (n = 16) | C (n = 15) | D (n = 4) |
| Lactic acid[b] | 0.913** | 0.934** | 0.802** | 0.273 |
| Acetic acid[b] | 0.623 | 0.311 | 0.861** | 0.451 |
| Butyric acid[b] | 0.374 | 0.104 | 0.461 | 0.990** |

[a] 0.1N NaOH ml/silage DM.
[b] % silage DM.
** $p < 0.01$.

to assess the silage quality based on the end products of fermentation such as lactic acid, VFA and ammonia. The possibility of using buffer index curve for silage evaluation is supported by the chemical compositions and Flieg's score of each group (table 1) and the correlation between the concentrations of acids and the peak values of buffer index curve (table 2).

The silage quality is generally used to indicate the success of fermentation and not to evaluate the feeding value of the silage. However, the fermentation quality and nutritional value of the silage are usually highly correlated because a poor fermentation can result in great loss of the digestible nutrients (Sutoh et al., 1970; Uchida and Sutoh, 1971, 1973). Although the nutritional values of silages were not determined in this study, it is assumed that the A and B group silages were better nutritional quality as well as better fermentation quality than the C and D group silages. In conclusion, the silages may be devided into 4 groups by the buffer index curve; two types of lactate silage depending upon the wilted extent, one medium quality silage, and one poor quality of butyrate silage.

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