

# THE RELATIONSHIP BETWEEN BUFFER INDEX CURVE AND FERMENTATION QUALITY OF SILAGE

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## Summary

The purpose of this study was to investigate the possibility of using the buffer index curve as a criterion of silage quality evaluation. The buffer index (or buffer capacity,  $\beta$ ) value is the amount of 0.1N NaOH consumed in titrating from the beginning to the end of the chosen pH step. This value equals the slope of the titration curve at the chosen pH range ( $\beta = \Delta \text{NaOH} / \Delta \text{pH}$ ).

There were two patterns of buffer index curve. Poorly preserved silages had a peak at pH 5.0, whereas with well preserved silages the peaks tended to rise to the pH 3.75 or 4.00. Well preserved, wilted silages with high pH also appeared to have peak values at pH 3.75 or 4.00. There was a high positive correlation between the concentration of lactic acid and peak value of good quality silage ( $r = 0.994$ ,  $p < 0.01$ ). Also a high positive correlation ( $r = 0.899$ ,  $p < 0.001$ ) was found between the sums of concentration of acetic and butyric acids and the peak values of poor quality silages.

The results of these experiments verified the value of the buffer index method as a criterion of silage quality evaluation.

(Key Words: Acetic Acid, Buffer Index Curve, Butyric Acid, Dissociation Constant, Lactic Acid, Silage Quality Evaluation)

## Introduction

The buffering capacity of plant material has been used to predict the possibility of satisfactory silage fermentation. McDonald and Henderson (1962) and Playne and McDonald (1966) demonstrated that after ensiling there is generally a two- to threefold increase in total buffering capacity from pH 4 to 6 due to the presence of organic acids. The buffering capacity of resulting silage, however, does not illustrate clearly the type of silage fermentation.

The theory of buffer action established by Van Slyke, quoted by Perrin and Dempsey (1973), predicts that the presence of Brønsted acids in a solution produces a peak in the buffer index curve; the position of which is determined by its dissociation constant, and the height of which is determined by its concentration in the solution. The end products of silage fermentation are usually complex mixtures of weak Brønsted acids, such as carboxyl group of amino acids, organic

acids and ammonium ion. The most important organic acids in a silage are lactic, acetic and butyric acids, which have  $pK_a$  values in the pH range of 3.5 to 5.5. The buffer index curve of a silage extract in this pH range thus contains information on the organic acids and the fermentation quality.

Green tea and flavoring foods have been analyzed by the buffer index curve, and in certain cases the results have suggested its practical use in quality evaluation (Tsuji, 1982; Tsuji and Takeo, 1983, 1985). The objective of this study was to consider the possibility of using the buffer index curve as a criterion of silage quality.

## Materials and Methods

### Experiment 1

Lactic, acetic and butyric acid solutions with known compositions were prepared to investigate the peak position of their buffer index curve. Each acid was prepared at concentrations of 0.4, 0.7 and 1.0M and 20 ml was titrated with 1.0N NaOH until the pH increased to 7.0. In a second series of experiments the effect of concentration on the buffer index curve was carried out with variable ratios between lactic

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acid and VFA. Initial pH and all further measurements taken during titration were recorded after 3 min. The buffer index curve is 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 ( $\beta = \Delta \text{NaOH} / \Delta \text{pH}$ ). That is, in this experiment, the buffer index value was the amount of 1.0N NaOH titrated in each 0.25 pH step.

### Experiment 2

Italian ryegrass (*Lolium multiflorum* Lam.) was ensiled at Okayama University in various conditions to produce stable and unstable silages which were used to investigate the possibility of using the buffer index curve as a criterion of silage quality evaluation. The silos were opened after 90 days and then top and bottom 5 cm of each silage were discarded before sampling. Each sample was collected and frozen until required for analysis. Silage quality was determined by measuring the pH value, the composition of VFA by gas chromatography (Uchida and Hayashi, 1985), lactic acid content by the method of Barker and Summerson (1941), and the proportion of volatile basic nitrogen (VBN) by semi-micro vacuum distillation (Ohyama, 1971).

Fresh silage (10g) was suspended in 150 ml distilled water and stirred with a magnetic stir bar for 10 min. The water extract was titrated to pH 2.5 with 0.1N HCl, and then titrated to pH 10 with 0.1N NaOH. The buffer index value was determined from the amount of 0.1N NaOH consumed in titration of each 0.5 pH step.

## Results and Discussion

### Experiment 1

The buffer index curves of lactic, acetic and butyric acid solutions are shown in figure 1. The pKa values of these acids are numerically equal to the pH at the peak of buffer index curve (Perrin and Dempsey, 1973). However, the peak positions of lactic acid (pKa = 3.86), acetic (pKa = 4.76) and butyric (pKa = 4.82) acids were at pH 4.00, 4.75 and 5.00, respectively. Even though the peak positions of the buffer index curves were different to the pKa values due to the effect of ionic strength, the peak heights were proportional to the concentrations of each acid.

The mixed solution containing equal propor-

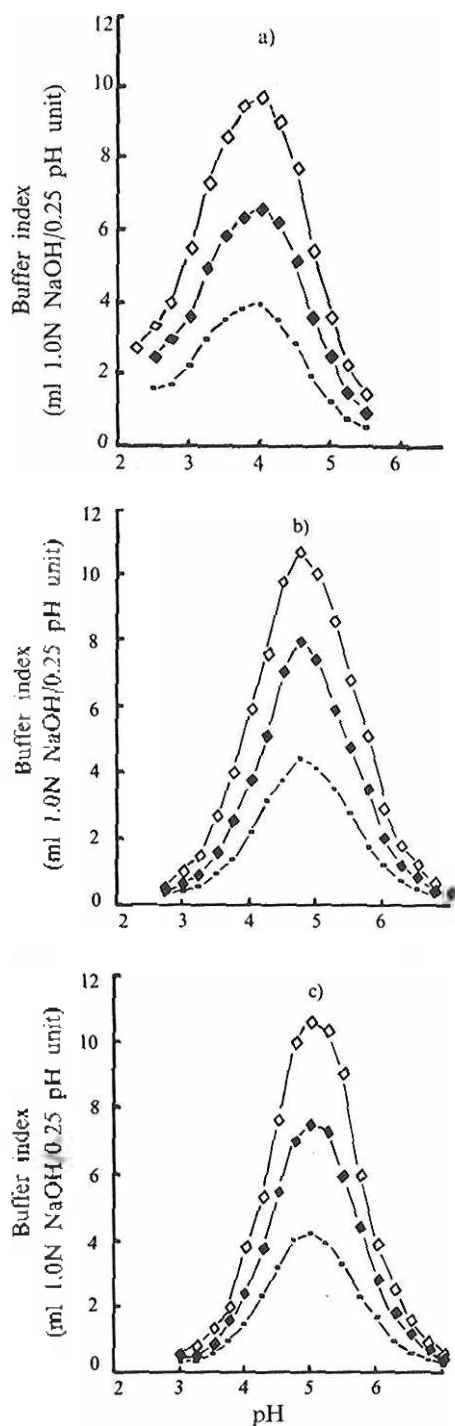


Figure 1. Buffer index peaks by titration of organic acid solutions with known compositions, each 20 ml, with 1.0N NaOH. (a) Lactic acid, (b) Acetic acid, (c) Butyric acid. ( $\diamond$ ) 1.0M; ( $\blacklozenge$ ) 0.7M; (—) 0.4M.

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tions of 0.4M of three acids showed only one peak at pH 4.75 (figure 2). The peak height was nearly equal to the sum of the buffer index value of each acid at pH 4.75. This result shows that the proportion of main VFA, which have similar pKa values, to lactic acid is the main factor relative to the position of peak being at 4.75 and its height in the mixed solution.

The effect of acid concentration on buffer index curve is shown in figures 3 and 4. Acetic acid was used as a representative of VFA presented in silage. When the concentration of lactic acid was more than 1.8 times the concentration of acetic acid, the peaks of the buffer index curves were at pH 3.75 or 4.00. On the other hand, when the concentration of acetic acid was more than 2.5 times the concentration of lactic acid, the peak of the buffer index curve was at pH 4.75. These results confirmed the theory of Van Slyke (Perrin and Dempsey, 1973) and suggested the possibility of applying this method to silage quality evaluation.

### Experiment 2

The chemical compositions of silage samples are shown in table 1 and typical buffer index curves of good and bad quality silages are illustrated in figure 5. The steep rise of the curve at both ends, beyond approximately pH 3 or 11, is caused by solvent water which has a large buffering capacity at these pH ranges (Perrin and Dempsey, 1973). In the case of well preserved silage, the peaks of the buffer index curves were

at pH 4.00. The silages of B group had a high pH value of 5.04 and the same peak position at pH 4.00. The chemical composition of the silages of B group agreed with a study (Uchida et al., 1989) in which wilting of crops prior to ensilage decreased the lactic, acetic and butyric acid contents and resulted in good preservation of silages at relatively higher pH values. Irrespective of wilting, a high positive correlation ( $r = 0.994, p < 0.01$ ) between the concentrations of lactic acid and the peak values of the buffer index curve was found in well preserved silages (table 2). These results can solve the problem of being unable to use pH as the sole criterion for assessing wilted silage fermentation.

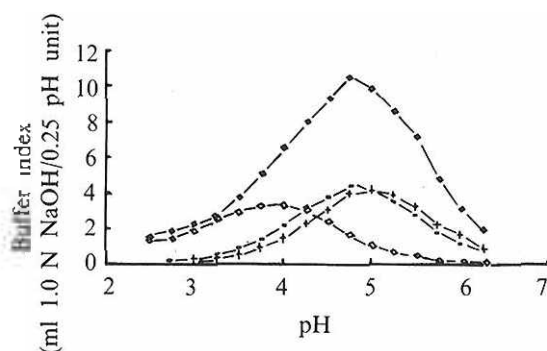


Figure 2. Buffer index peaks by titration of mixed solution (20 ml), each 0.4M, with 1.0N NaOH.

(◆) Mixed solution of lactic acid 0.4M (◇), acetic acid 0.4M (—) and butyric acid 0.4M (+).

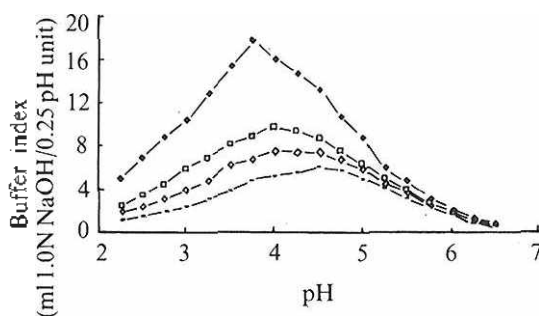


Figure 3. Concentration effects (Lactic acid: Acetic acid) on the buffer index curves. Mixed solutions (each 20 ml) were titrated with 1.0N NaOH.

(—) 0.4M: 0.4M; (◇) 0.7M: 0.4M; (□) 1.0M: 0.4M; (◆) 2.0M: 0.4M.

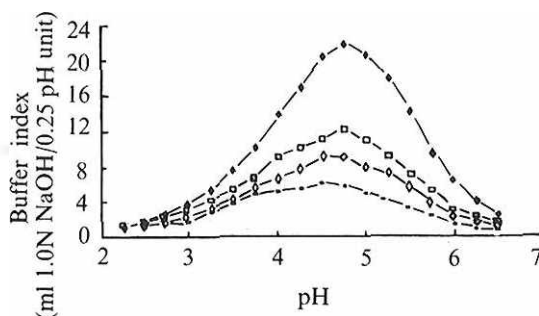


Figure 4. Concentration effects (Lactic acid: Acetic acid) on the buffer index curves. Mixed solutions (each 20 ml) were titrated with 1.0N NaOH.

(—) 0.4M: 0.4M; (◇) 0.4M: 0.7M; (□) 0.4M: 1.0M; (◆) 0.4M: 2.0M.

TABLE 1. CHEMICAL COMPOSITIONS OF SILAGES FOR DETERMINING BUFFER INDEX CURVES

	Silage		
	A (n = 2)	B (n = 2)	C (n = 9)
DM (%)	17.07 ± 0.25 <sup>c</sup>	40.00 ± 0.09	13.05 ± 0.69
pH	4.04 ± 0.07	5.04 ± 0.14	5.36 ± 0.40
Lactic acid <sup>a</sup>	12.03 ± 0.76	4.75 ± 0.37	0.10 ± 0.07
Butyric acid <sup>a</sup>	0.07 ± 0.04	0.46 ± 0.10	5.40 ± 1.05
Acetic acid <sup>a</sup>	0.73 ± 0.67	0.65 ± 0.24	5.71 ± 2.29
VBN <sup>b</sup>	5.74 ± 0.91	4.24 ± 0.92	34.14 ± 10.11

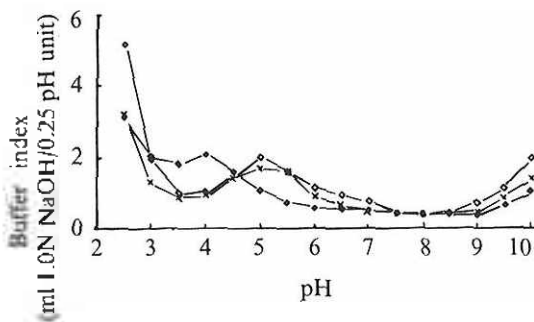
<sup>a</sup> % silage DM.<sup>b</sup> Volatile basic nitrogen (% total nitrogen).<sup>c</sup> Mean ± SD.

Figure 5. Typical buffer index curves of silages. Water extracts (each 20 ml) titrated with 0.1 N NaOH.  
 (◆): Well preserved silage;  
 (◇) and (×): Poorly preserved silages.

TABLE 2. CORRELATION COEFFICIENT BETWEEN THE CONCENTRATION OF ACID AND THE PFAK VALUE OF THE BUFFER INDEX CURVE

	Peak value <sup>a</sup>	
	Stable silage (n = 4)	Unstable silage (n = 9)
Lactic acid <sup>b</sup>	0.994**	
Acetic acid <sup>b</sup>		0.961***
Acetic + Butyric acid		0.899***

<sup>a</sup> 0.1N NaOH ml/silage DM.<sup>b</sup> % silage DM.

\*\* p &lt; 0.01; \*\*\* p &lt; 0.001.

The silages of the C group had high pH values, low contents of lactic acid and high

contents of butyric acid. As expected, for these silages the peak occurred at pH 5.00 and there were high positive correlations between the peak values of the buffer index curves and the acetic acid contents ( $r = 0.961$ ,  $p < 0.001$ ), and the sum contents of acetic and butyric acid ( $r = 0.899$ ,  $p < 0.001$ ). Moisio and Heikonen (1989) suggested five equations for determining five components by using its pKa ranges. However, they did not report a change in the peak position of the buffer index curve in the pH range 3.5 to 5.5, relative to the difference in concentration between lactic acid and the other organic acid.

This method has advantages over the distillation method of Flieg (1971) and chemical analysis by gas chromatography in requiring less capital and time. The ratio between lactic and other organic acids from figures 3 and 4 may provide additional information for separating levels of silage quality. Further studies should be carried out with silage to compare the buffer index curve method with other evaluation methods.

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