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## Note

# Determination of lactic and acetic acids in silage extracts by analytical isotachophoresis

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Lactic and acetic acids are usually the major fermentation products present in silage and their determination is therefore of importance in assessing silage quality. The first attempts at determining these acids in silage were made by Flieg[1] and Lepper[2], who made use of the method of half-distillation and determined acetic, butyric and lactic acids. These methods suffer from serious errors, but, they have still been used in routine analyses of silages. In the past decade a number of gas chromatographic methods for the determination of lactic acid in silages and other biological materials have been described[3–9]. However, the pre-treatment of the sample prior to gas chromatographic analysis is time consuming and rather complicated, and the question of interferences due to other substances present in silage has not yet been solved successfully[8].

We report here some results showing that high-speed isotachophoresis[10,11] can be used successfully for the determination of lactic and acetic acids in silage extracts. This technique does not require any preliminary treatment of the silage extract, and the analysis of a sample of 2–5 $\mu$l is complete in 10 min.

## EXPERIMENTAL

The system of 0.012 *M* hydrochloric acid and 0.02 *M* urotropin at pH 4.9 without any further admixture was selected as the leading electrolyte and 0.01 *M* sodium hydrogen carbonate as the terminator, as these were the most suitable of several electrolyte systems that were tested. All of the chemicals used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia). The separations were carried out on an isotachophoregraph of the authors' own design[9,10] at a constant current of 240 $\mu$A and 22°. Aqueous extracts were prepared from silages (Bioveta, Nitra, Czechoslovakia) by the standard procedure. The samples of silage extracts were injected with a Hamilton microsyringe without any preliminary treatment, the volumes injected being 2.60 $\mu$l. Aqueous solutions of 0.01 *M* oxalic, formic, acetic, propionic and butyric acids and a 0.01 *M* solution of lithium lactate served as standard solutions, and the amounts injected for preparation of the calibration graphs were 2–16 $\mu$l.

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## RESULTS AND DISCUSSION

The simultaneous determination of lactic, acetic and volatile fatty acids in aqueous silage extracts by means of isotachophoresis is not without problems. The mixture concerned contains weak or medium-strong organic acids that differ only slightly in the size of their molecules and in their p$K$ values and, consequently, also in their effective mobilities. Several electrolyte systems with pH values in the range 3.8–6.2 were therefore tested for their suitability for the isotachophoretic analysis of silage extracts. A leading electrolyte of pH 4.9 and with chloride as the leading anion and urotropine as the buffering counter ion, and hydrogen carbonate as the terminating ion, were selected as the most suitable. In this system, lactic and acetic acids are separated completely from oxalic, formic and higher volatile fatty acids that may be present in the extracts. The separation is shown in Fig. 1 for the analysis of a model mixture containing oxalate, formate, lactate, acetate, propionate and butyrate each at a concentration of 0.01 $M$. Propionic acid at this pH is not well separated from butyric acid (or from higher fatty acids) and forms a mixed zone, but it is well separated from both lactic and acetic acids.

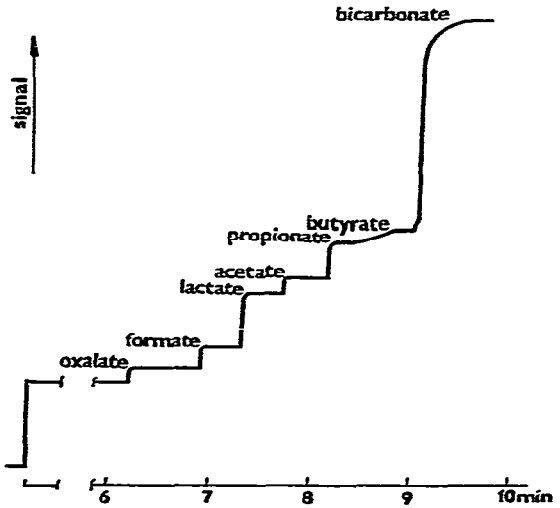


Fig. 1. Analysis of a model mixture containing oxalic, formic, lactic, acetic, propionic and butyric acids, each at a concentration of 0.01 $M$. The volume of the mixture injected was 2.60 $\mu$l. The leading electrolyte was 0.012 $M$ HCl and 0.02 $M$ urotropine, pH 4.9; the terminator was 0.01 $M$ $NaHCO_3$. The driving current was 240 $\mu$A.

For quantitation, direct comparison of the lengths of the zones of a sample and those of a standard solution is used, for which purpose a calibration graph is advantageous. Fig. 2 shows calibration graphs for amounts of the acids *versus* the lengths of their zones (the chart speed was 3 cm/min). It can be seen that the graphs are satisfactorily linear. By considering a 1-mm zone in the column, *i.e.*, about a 2-sec interval in the record (the rate of travel of the zones was about 4 cm/min) as the practical limit for quantitation, we can calculate that the smallest amount that

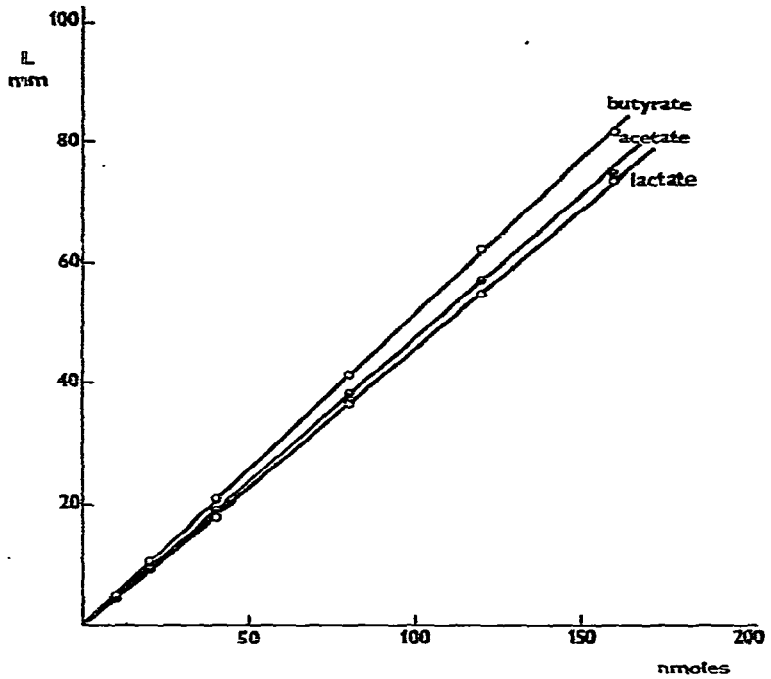


Fig. 2. Calibration graphs for lactate, acetate and butyrate. *L* mm is the length of the zone in the isotachophoregram (chart speed, 3 cm/min). For separation conditions, see Fig. 1.

can be quantitated is 2 nmole. For a 10-$\mu$l sample this corresponds to a concentration of $2 \cdot 10^{-4}$ *M*. The reproducibility of the method was determined by replicate analyses, the coefficient of variation being 2.3%.

Lactic and acetic acids are the most important organic compounds for the determination of the quality of silage extracts. Silage of high quality, in which proteolysis is significantly supressed, always contains, in addition to lactic acid, only acetic acid and very small amounts of propionic and butyric acids. In well fermented silage, two thirds of the above acids should be lactic acid, at the most one third acetic acid, and butyric acid should not be present. The degree of proteolytic decomposition is expressed in terms of an increased content and variety of higher fatty acids produced by the deamination of amino acids.

Figs. 3 and 4 show examples of the records of isotachophoretic analyses of extracts of two different silages. The silage extract represented in Fig. 3 contained 0.0410 *M* lactic acid and 0.0110 *M* acetic acid. This extract represents silage of very good quality, in which higher fatty acids are present in trace amounts only. The other extract (Fig. 4) contained lactic acid in a concentration of 0.0176 *M*, acetic acid in a concentration of 0.0150 *M* and butyrate (plus higher acids if present) in a concentration of 0.0012 *M*. In addition, the silage contained formic acid as a preservative.

It can be seen that high-speed isotachophoresis enables one to determine lactic and acetic acids in silage extracts within 9 min without preliminary treatment of a

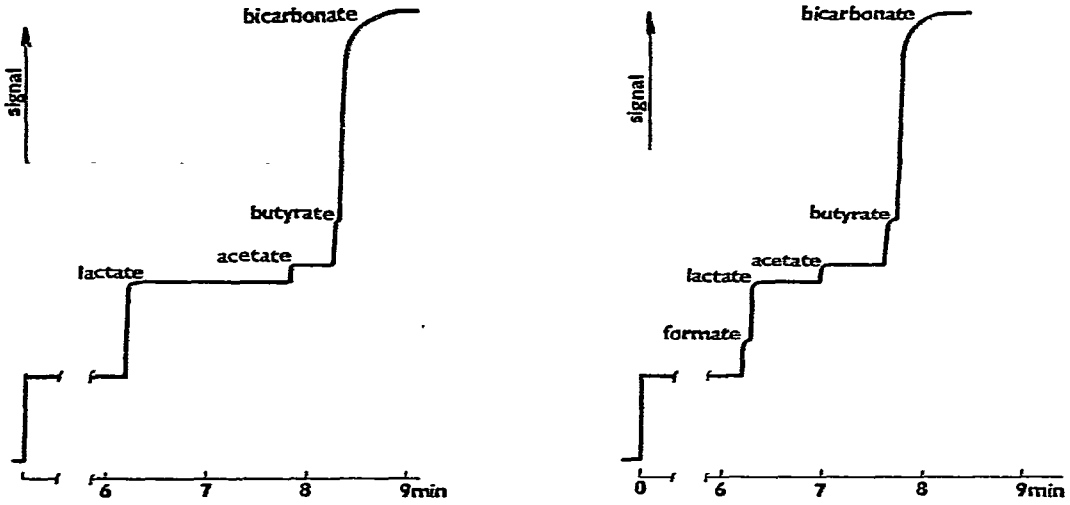


Fig. 3. Isotachophoregram of a silage extract containing 0.0410 *M* lactic acid and 0.0110 *M* acetic acid. For separation conditions, see Fig. 1.

Fig. 4. Isotachophoregram of a silage extract containing 0.0176 *M* lactic acid, 0.0150 *M* acetic acid and 0.0012 *M* butyric acid. In addition, the silage contained formic acid as a preservative.

sample. Moreover, the presence of other acids such as formic, propionic and butyric acids can be established and their concentrations determined, the lowest concentration that can be determined being about $1 \cdot 10^{-4}$ *M*.

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