A Gas Chromatographic Method for the Determination of Acetic and Lactic Acid in Rye Sour[1]

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ABSTRACT

A rapid gas chromatographic method for the determination of acetic and lactic acid in rye sour is described. A neutralized aqueous extract of rye sour is prepared, and the acetic acid and ethanol contents are determined. Simultaneously, an aliquot of the extract is oxidized by excess of potassium permanganate in the presence of sulphuric acid. Acetic acid in the oxidized sample is determined. The lactic acid content is obtained when the acetic acid and ethanol contents in the original extract are subtracted from the acetic acid content in the oxidized extract. The described method cuts down the time of one complete analysis to one quarter of the time required by currently used esterification procedures.

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One of the most important stages in the production of rye and rye-wheat bread is the fermentation of rye sour (Rohrlich 1961, Schulz 1966). Flavor, aroma, and texture of the final loaf of bread are to a great extent dependent on the characreristics of the fermented sour.

During several steps of sour fermentation, the microflora preexisting in rye sour are allowed to multiply and the optimum amounts and ratio of yeasts and of homofermentative and heterofermentative lactic acid bacteria are obtained. These symbiotically coexisting microorganisms produce the desired metabolites: carbon dioxide, lactic and acetic acids, ethanol, and some other compounds.

The lactic and acetic acids produced by lactic acid bacteria are of great importance in the technological process of rye-wheat bread production. They provide the optimum pH for amylolytic enzymes. They positively affect the swelling of colloid particles, especially proteins, and consequently, the rheological properties of the dough. They suppress the growth of undesirable microflora in sour. Of particular importance is the significant role they play in the formation of final bread aroma and flavor.

A rapid and reliable method for determining lactic and acetic acids in rye sour is therefore very important. Such a method should allow the analysis of either parallel samples or a sequence of samples.

Various methods for determining organic acids in food materials have been described. The usual method of determination of lactic acid consists in moderate oxidation of a sample followed by titration or colorimetric determination of produced acetaldehyde (Brümmer and Klempin 1968, Galal et al 1978). The oxidation should be performed carefully; otherwise losses of acetaldehyde and consequently of lactic acid may occur.

Recently, enzymatic methods for determination of acetic and lactic acids have been proposed (Rabe 1977, Wutzel 1976). Acetic and lactic acids can also be determined by an AACC distillation procedure.

For determination of the whole spectrum of organic acids in food products or for parallel assessment of several acids, one of the chromatographic methods is usually employed. The classic, time-consuming column chromatography (Marková and Hampl 1969) has been gradually replaced by high performance liquid chromatography (Palmer and List 1973, Wildanger 1975). However, gas chromatography is still frequently used.

Gas chromatography is a suitable technique for relatively quick and simple determination of lower nonsubstituted monobasic acids (Doelle 1969, Galal et al 1978). Special conditions for the determination of free lactic acid have been described (Atzeni et al 1972). However, polybasic and substituted acids, eg lactic acid, are usually converted into their more volatile derivatives, such as methylesters (Drucker 1970), propylesters (Staruszkiewicz 1969), and butylesters (Reagan et al 1971) or silyl compounds (Fernandez-Flores et al 1970). This is a rather complicated and time-consuming procedure. Therefore, a new rapid method for determination of acetic and lactic acids has been developed.

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MATERIALS AND METHODS

Principle of the Method

The method is based on the conversion of lactic acid to acetic acid by oxidation with excess of potassium permanganate in the presence of sulphuric acid (Davies 1971). Nonoxidized as well as oxidized samples are analyzed by gas chromatography. The lactic acid content is obtained from the difference between the acetic acid in the oxidized sample and the acetic acid and ethanol in the nonoxidized sample.

Samples of Sour

Sours were either taken from the production line in an industrial bakery or prepared in the laboratory, with sour from the bakery serving as an inoculum (ie, a portion of the ripe sour used for making a new one). Samples of the sour were immediately frozen and kept at −18°C until analyzed. During two weeks of storage under these conditions no changes in the organic acids content occurred.

Preparation of the Extract

A sour sample (10 g) was homogenized with 25 ml of water in a laboratory mixer and the suspension centrifuged at 3,000 rpm for 30 min. Acids in the supernatant were neutralized with 2 *N* sodium hydroxide, and enzymes were inactivated by boiling the extract for 5 min. After cooling, the sample was centrifuged at 40,000 rpm for 20 min. A considerable amount of precipitated protein was removed. The clear supernatant was transferred into a 50-ml volumetric flask and made to volume.

Preparation of Samples for the Analysis

The nonoxidized sample was prepared by mixing 10 μl of *n*-propanol with 10 ml of the extract.

For the oxidized sample, an aliquot (10 ml) of the extract was pipetted into a 100-ml flask; the required amount (usually 0.8 g) of solid potassium permanganate was added and the mixture agitated for 30 sec. Then, 6 ml of 4*N* sulfuric acid was added and the mixture was kept boiling under a condenser for 10 min. The sample was cooled; the condenser was flushed with a few milliliters of water; and 10 μl of *n*-propanol was added to the flask. The mixture, containing a dark brown precipitate of manganese dioxide, was filtered into a well-tight sample bottle. Analyses of standard samples have shown that quantitative oxidation of lactic acid and ethanol to acetic acid requires a fourfold excess of potassium permanganate.

Gas Chromatographic Analysis

Analyses were performed with a Perkin-Elmer model F-7 gas chromatograph equipped with flame ionization detector (sensitivity 1/8). A steel column (1 ml long and 3 mm ID) packed with Porapak Q 120–150 mesh was held at a temperature of 200° C. Nitrogen (20 ml/min) was used as carrier gas. Injection volume was 0.9 μl; *n*-propanol was used as the internal standard.

Acetic acid and ethanol contents in the original extract were determined by analysis of the nonoxidized sample. These two values were subtracted from the acetic acid content in the oxidized sample, and the lactic acid content was thus obtained. Examples of chromatograms are shown in Fig. 1.

Peaks for *n*-porpanol in the chromatograms are of different heights because of the larger volume of the oxidized sample. No ethanol peak, of course, occurs in the chromatogram of the oxidized sample. If nonquanitative oxidation of lactic acid occurs, acetaldehyde is partially formed, and its peak would appear on the declining branch of the peak for water. Water gave a slight response in the FID detector at high sensitivities.

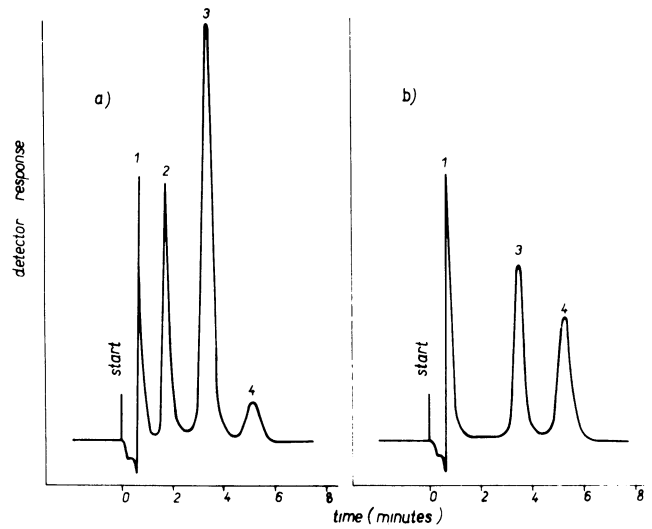

Fig. 1. Gas chromatograms of rye sour extracts: **a,** nonoxidized extract; **b,** oxidized extract. 1 = water, 2 = ethanol, 3 = *n*-propanol, 4 = acetic acid.

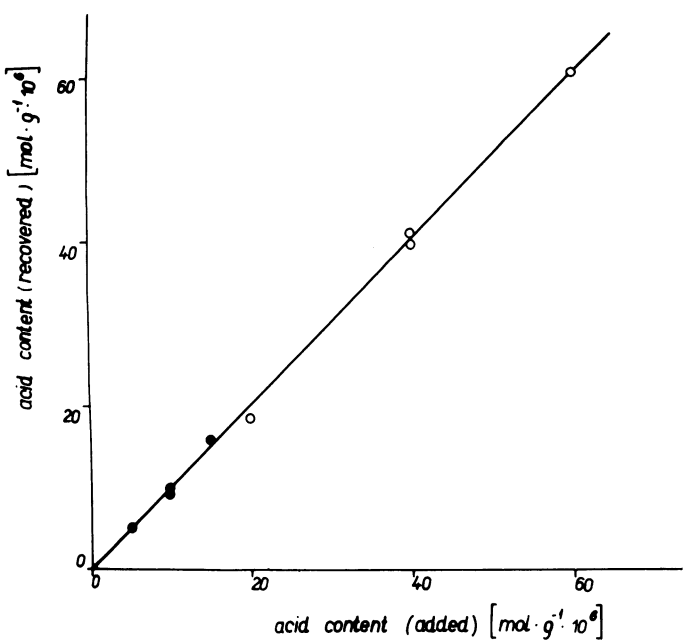

Fig. 2. Recovery of acetic acid (●, $\bar{r} = 0.978$) and lactic acid (o, $\bar{r} = 0.980$).

Calibration

Sets of standard solutions of 2–50 m*M* sodium acetate and 2–15 m*M* ethanol were prepared. Ten μl of *n*-propanol was added to 10 ml of the standard solutions, and the samples were analyzed under the described conditions. The peak areas of standard and test samples were calculated relative to the peak for *n*-propanol. No significant differences were found in the analyses of standard solutions of actetic acid, sodium acetate, and oxidized solutions of lactic acid of equal molarities.

RESULTS AND DISCUSSION

Effect of Oxidizable Compounds

In an aqueous extract of rye sour, many oxidizable compounds besides ethanol and lactic acid are present. Knowledge of whether and to what extent the oxidation of these compounds could affect the conditions described above, saccharides (eg, glucose and maltose) as well as acids of the Krebs cycle (eg, citric and tartaric acids) are oxidized to carbon dioxide and water. These compounds therefore do not directly interfere with the lactic acid determination. Their presence, however, requires a higher dosage of oxidizing agent.

Under oxidation, some amino acids are partially decomposed to acetic acid. Nevertheless, tested samples of alanine and leucine yielded a reaction of only about 10%. Rye sour contains a small amount of free amino acids (Rohrlich and Hertel 1966) relative to its lactic acid content (Rabe 1977). Considering these facts, we can ignore the error caused by the oxidation of amino acids.

Recovery

The described method has been verified by a standard addition procedure. Different amounts of acetic and lactic acids were added to samples of rye sour in the mixer, and samples with and without addition were analyzed in parallel. The recovery value was obtained from the difference in the acid contents in the samples. Results are summarized in Fig. 2. The straight line corresponds to 100% recovery (or r=1.0). Mean r values of 0.978 and 0.980 for the recovery of acetic and lactic acid, respectively, were obtained.

Statistical evaluation

Thirteen samples of the same sour were analyzed. Values obtained for acetic acid ranged from 0.84 to 0.95 mg/g and those of lactic acid from 5.33 to 6.21 mg/g. Results of the statistical evaluation are summarized in Table I. Coefficients of variation of about 4% are satisfactory for routine applicaiton of the method.

Example of Application

In a production line, the process of rye sour fermentation is repeated in such a way that two-thirds of the sour is used for dough mixing and one-third for making a new sour. The fermentation time of repeated rye sour is usually 3 hr. One of the most important problems in bread making is the spanning of breaks that occur in a continuous production process (eg, from one-shift production, weekends, etc).

Properties of fully fermented sour are determined by three main factors: proportion of inoculum, temperature and fermentation time. In other words, the optimum fermentation time of sour can be prolonged by changing the proportions of inoculum and

TABLE I
Statistical Evaluation of the Method

Parameter	Acetic Acid	Lactic Acid
Arithmetic mean (mg/g)	0.895	5.790
Standard deviation (mg/g)	0.037	0.265
Coefficient of variation (%)	4.12	4.58
Standard deviation of arithmetic mean (mg/g)	0.010	0.073
Confidence interval[a] (mg/g)	0.895 ±0.022	5.790 ±0.159
Limit of determinability[a] (mg/g)	0.081	0.577

[a]For confidence coefficient 0.95.

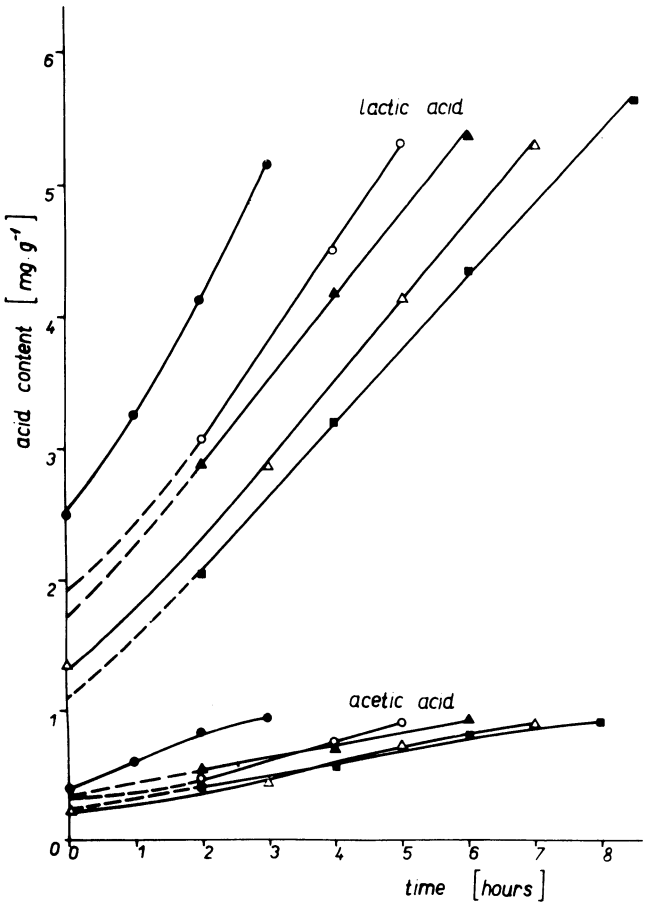

Fig. 3. Time dependence of acetic and lactic acid contents in sour fermented under different conditions. Optimum proportion of inoculum, temperature, and fermentation time for respective courses of sour fermentation: ● = 1:3, 30°C, 3 hr; o = 1:5, 27°C, 5 hr; ▲ = 1:6, 26 °C, 6 hr; Δ = 1:7, 25°C, 7 hr; ■ = 1:8, 25 °C, 8 hr.

temperature.

Maturity of sour is indicated, among other characteristics, by the content and ratio of acetic and lactic acids. Using these variables, the end of the fermentation period can be ascertained. Fig. 3 demonstrates the time-dependence of acetic and lactic acids in sours, showing the optimum fermentation time for different combinations of inoculum proportion and temperature. By changing the proportion of inoculum and the temperature, equal amounts of acetic and lactic acids in sours can be obtained with different optimum fermentation times.

With a slight modification, the described procedure for determining acetic and lactic acids in rye sours can also be applied to flour and bread.

CONCLUSION

The proposed method has proved to be suitable for studying the content of acetic and lactic acids in rye sours and other cereal materials. The method provides reproducible results in a relatively short time, which is its main advantage. The total time of analysis for one sample is about 2.5 hr, but parallel analyses of four samples can be performed in less than 4 hr. By contrast, gas chromatographic analysis by the esterification procedure requires at least 8 hr and liquid column chromatography more than 24 hr for a single sample. Results of the recovery test and of statistical evaluation show the described method to be satisfactory for routine application.

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