Paper-chromatographic identification of organic acids resulting from fermentations

Organic acids, as primary and secondary products of fermentations, were investigated by paper chromatography. The application of this technique to the study of fermentations is vast and very important. Industrial fermentations are generally controlled by titration of the acid. In this way the total acid content is determined, without any indication as to which acids are responsible for the acidity. In research work paper chromatography can contribute much to the study of the biochemistry of each process, since it enables the order to be determined in which the acids are formed during fermentation.

Materials and methods

Ascending chromatography[1] on Whatman No. 1 paper was the method employed. The solvent that gave best results was propyl alcohol–ammonia (70:30 v/v) (ISHERWOOD[2]), development being carried out for 7 h at room temperature. For the detection of the spots bromocresol green solution was used according to KENNEDY[3], except that water was replaced by alcohol, and spraying was followed by 10 min heating at 80°.

The identification was made by comparison of the R_F values with standard 1% solutions of organic acids and, in some cases, by specific reactions. Volatile acids were used in the form of their ammonium salts[3]; because of the instability of these salts the preparation, development and detection of the chromatogram should be made on the same day.

The fermentations were carried out following the techniques and conditions described by PRESCOTT AND DUNN[4].

Results

Alcoholic, lactic, acetic and citric fermentations were tested in this investigation. Each mash was chromatographed before and after fermentation, together with the standard organic acids (Figs. 1 and 2).

The raw materials, conditions and results of each process are set forth in Table I.

Discussion

Alcoholic fermentation. Succinic and acetic acids were identified as by-products of the process. Regardless of the quality of the presence of raw material, the presence of succinic acid was observed also in some initial mashes of molasses, which could certainly be ascribed to a beginning of auto-fermentation.

Lactic fermentation. When skim milk was used as raw material both lactic and

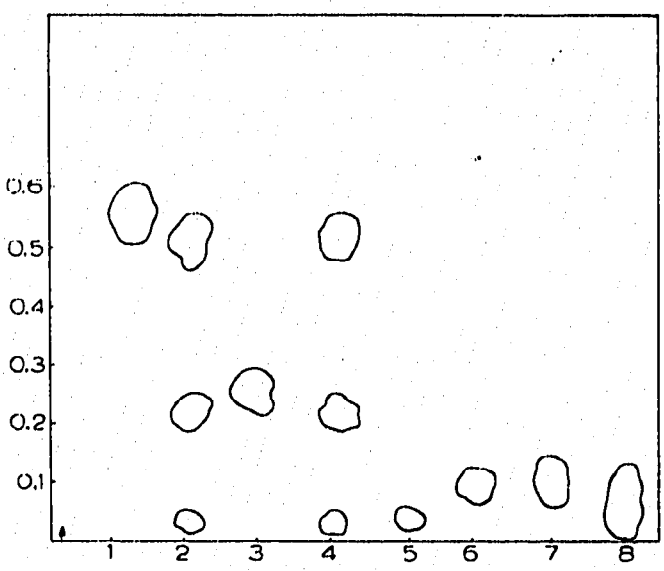

Fig. 1

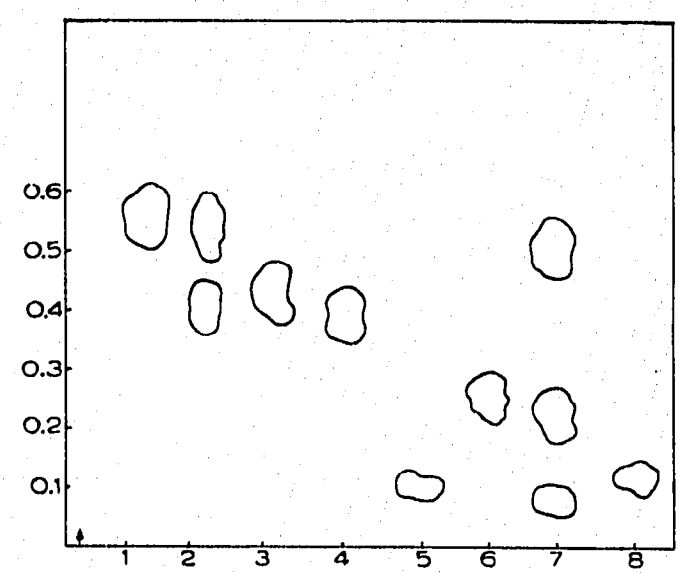

Fig. 2

Fig. 1. (1) acetic acid; (2) alcoholic fermentation(molasses); (3) succinic acid; (4) alcoholic fermentation (glucose); (5) molasses mash; (6) citric acid; (7) citric fermentation; (8) oxalic acid.
Fig. 2. (1) acetic acid; (2) lactic fermentation (skim milk); (3) lactic acid; (4) lactic fermentation (molasses); (5) tartaric acid; (6) succinic acid; (7) acetic fermentation; (8) malic acid.

acetic acids were identified. When molasses mash was used only lactic acid appeared at the end of the fermentation.

Acetic fermentation. Acetic, tartaric and succinic acids were identified and these are therefore responsible for the acidity values found in titrations carried out during fermentation. Since the R_F value of tartaric acid is similar to those of citric, oxalic and malic acids, the detection was carried out with a solution of silver nitrate (ISHERWOOD[2]), which gives a black spot for tartaric acid and white ones for the three others.

TABLE I

ORGANIC ACIDS IDENTIFIED IN FERMENTATIONS

Fermentation	*Raw material*	*Microorganism*	*Conditions*				*Acids identified*
			°Bx	*°C*	*pH*	*time*	
Alcoholic	molasses	*Saccharomyces cerevisiae* ATCC 764	17	25	4.5	48 h	acetic, succinic
Alcoholic	sucrose	*Saccharomyces cerevisiae* ATCC 764	12	25	4.5	48 h	acetic, succinic
Alcoholic	glucose	baker's yeast (Fleischmann)		25	4.5	48 h	succinic
Lactic	skim milk	*Lactobacillus casei* 7460		37	6.5	72 h	acetic, lactic
Lactic	molasses	*Lactobacillus casei* 7460	12	37	6.7	72 h	lactic
Acetic	ethyl alcohol	*Acetobacter suboxydans*		27		10 d	acetic, tartaric, succinic
Citric	sucrose	*Aspergillus niger* 1015		25	3.5	10 d	citric

Citric fermentation. Only citric acid was identified although oxalic acid frequently appears during the process. These two acids, the R_F values of which are very similar, can be distinguished by elution with pyridine and acetic anhydride according to FURT AND HERMANN[5], a red colour being obtained for citric acid and gas evolution for oxalic acid.

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Chromatography of neutral amino acids on columns of cellulose powder

In a previous paper[1] we described the fractionation of peptides in columns of cellulose powder using a volatile solvent, *viz.*, a mixture of ethyl alcohol and water. In spite of the great number of existing methods for the separation of amino acids we thought it would be interesting to apply this system to the fractionation of these acids.

Two sizes of columns were used in this work, smaller ones of 31 × 0.9 cm and larger ones of 100 × 0.9 cm. The columns were prepared by pouring into a glass tube a slurry obtained by suspending 1 part of cellulose powder (Whatman standard grade) in 3 parts of a mixture of ethyl alcohol and water of the appropriate concentration. After the cellulose powder had sedimented a pressure of 15 lbs. per sq. in. was applied. The columns were then washed under pressure with about 2 l of the ethyl alcohol–water mixture. These columns can be used many times, provided they are washed between runs with about 1 l of absolute ethyl alcohol and then equilibrated with the solvent to be used for the chromatography.

The columns were mounted on an automatic fraction collector and the flow was adjusted to 2.5 ml/h; 0.75 ml fractions were collected. Alternate fractions were analysed by a modification of the ninhydrin method of TROLL AND CANNAN[2] and by one-dimensional paper chromatography.