is of a complex type, and changes in sucrose content hereafter cannot be reasonably correlated with any single factor, though formation of F_2G is still the dominant feature.

Considering only plant transfructosidases, Edelman & Bacon (1951) could not observe the synthesis of glucofructosans from sucrose alone by the action of enzyme from the tubers of Jerusalem artichoke unless inulin was also present. Dedonder (1952), repeating these experiments, found, on the contrary, that sucrose could be acted upon independently and that in the presence of inulin higher oligosaccharides were produced and more rapidly. The dialysed enzyme from the agave stem does not contain chromatographically detectable polyfructosans or lower carbohydrate fragments, but is still able to build glucofructosans from the sucrose alone. Also, this activity is not enhanced by agave fructosan. So far as the agave enzyme is concerned, there seems to be no necessity, therefore, to assume that the enzyme can only act in a coupled system consisting of fructosans or intermediates as fructose donors and sucrose as the acceptor.

We are not in a position to say yet whether the *in vitro* formation of glucofructosans from sucrose by the action of agave enzyme is a step in the *in vivo* mechanism of the biosynthesis of the agave fructosans.

SUMMARY

1. The stem of *Agave vera cruz* contains a transfructosidase which builds up glucofructosans from sucrose with simultaneous release of glucose. With the progress in incubation, small quantities of fructose are released.

2. The enzyme has been partially purified and concentrated by precipitation with ammonium sulphate and dialysis.

3. No dialysable cofactors seem to be involved in the enzyme action.

4. The enzyme acts on sucrose as the single substrate without requiring the interaction of agave fructosans or intermediates. No higher homologues are formed from raffinose, which is, however, slightly hydrolysed during prolonged incubation with the enzyme.

5. Inorganic phosphate does not influence the enzyme action.

6. The conditions for optimum action are: pH 5.6–5.8; temperature 37° and a sucrose concentration of 30 %. The enzyme is inactivated at 55°.

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Gas-Liquid Partition Chromatography: the Separation and Micro-estimation of Volatile Fatty Acids from Formic Acid to Dodecanoic Acid

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James & Martin (1952) published a method for the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid, making use of gas-liquid chromatograms. In this method the mixture of the fatty acids dissolved in ether is brought on to a kieselguhr column, the liquid phase consisting of DC 550 silicone, containing 10 % of

stearic acid. By heating the column to 100° or 137° the fatty acids are volatilized, separated with the aid of a nitrogen current and titrated successively.

However, the authors do not describe how a mixture of the lower volatile fatty acids (formic up to valeric acid) and the higher volatile fatty acids

(hexanoic up to dodecanoic acid) can be quantitatively separated and determined in one process. By bringing the column immediately up to 137°, a temperature necessary for the analysis of hexanoic up to dodecanoic acid, a good separation of the lower fatty acids is impossible, while at a column temperature of 100° a separation of the acids C_6 up to C_{12} is impracticable. A quantitative determination of formic acid, as well as the higher volatile fatty acids requires, moreover, extra care when the acids which have to be brought on to the column are dissolved in a relatively large quantity of ether. For this a temperature of 150° is necessary, while, at the same time, air is drawn through the column for 15 min., which procedure, however, results in loss of formic acid (see Table 1).

It proved possible to separate the acids from C_1 up to C_{12} in one process by gradually increasing the temperature of the column from 100° to 150°, by which means first formic acid and then up to valeric acid are sharply separated and then hex-

anoic acid to dodecanoic acid also. If the ether, which may be present in large quantities, is removed by blowing instead of by suction, formic acid is prevented from escaping titration. Vapourizing the acids is next effected by bringing the chromatographic tube up to 100° and gradually raising the temperature to 150°. The chromatographic tube must be in an approximately horizontal position in the vapour jacket and need not be removed when the next analysis is begun (Gerritsma, 1954).

EXPERIMENTAL

Apparatus

The column is prepared according to James & Martin (1952), the same reagents being used. The apparatus described by James & Martin was slightly modified (see Fig. 1). The chromatographic tube has a small aperture *A* which can be closed with a rubber stopper, fixed with a clamp. Furthermore, the tube is widened at *B* in front of the packing but inside the heating jacket, forming a hollow with a capacity of 2 ml. if the chromatographic tube is slightly inclined. Under the heating jacket at *B* a micro-burner is placed. The metal jacket is heated by circulating glycerol from a thermostat at such a rate that the temperature at both ends of the tube does not differ by more than 1°. By regulating the thermostat the temperature of the heating jacket can be gradually raised.

Method

The determination is carried out as follows. The acids, dissolved in ether, are pipetted through the aperture *A* into the chromatographic tube. Because the tube is slightly inclined, the ether solution of the fatty acids collects in the hollow *B*. After rinsing with a few drops of ether through

Table 1. *Recovery of lower volatile fatty acids*

4.7 μ equiv. formic, 16.0 μ equiv. acetic and 11.6 μ equiv. propionic acid brought on column by heating for 15 min. at 150° (b.p. bromoform), or by heating for 15 min. at 100°, while air was sucked through.

	Recovery (%)		
	Formic acid	Acetic acid	Propionic acid
15 min. at 150°	79	—	—
	83	96	98
15 min. at 100°	97	100	100
	94	100	100

Fig. 1. Chromatographic tube with heating jacket and titration vessel. For explanation, see text.

Fig. 2. Separation of a mixture of 1–5 μ equiv. of each of the following acids: formic, acetic, propionic, *isobutyric*, *n*-butyric, *isovaleric*, *n*-valeric, hexanoic, heptanoic, nonanoic and decanoic acid. Liquid phase, stearic acid (10%, w/w) in DC 550 silicone; nitrogen pressure 55 cm. Hg; flow rate 50 ml./min. After 85 min. the time scale was changed. After 35 min. the temperature of 100° was raised, after 83 min. 137° was reached, 30 min. later 150°, the temperature remaining at this level till the end of the analysis. Recovery: $C_1=97\%$, $C_2=98\%$, $C_3=101\%$, *iso*- $C_4=105\%$, $C_4=101\%$, *iso*- $C_5=100\%$, $C_5=101\%$, $C_6=102\%$, $C_7=97\%$, $C_8=107\%$, $C_9=108\%$, $C_{10}=102\%$.

A, the latter is closed, the nitrogen stream and the titration cell are connected and the ether is evaporated, first at room temperature for 20 min., afterwards by gentle heating for 10 min. with a micro-burner under the heating jacket where B is situated. Then the thermostat is switched on, bringing the temperature in the tube up to 100° by means of the glycerol. The acids in B evaporate as a consequence and at the same time the separation commences, at first of the lower fatty acids only, later of the higher fatty acids also. The acids passing through the titration cell are titrated. After the lower fatty acids up to *n*-butyric acid have been separated and estimated, the temperature of the glycerol is gradually raised to 150°, so that the remaining acids can be successively titrated. Raising the temperature is carried out in such a way that a temperature of 137° is reached when heptanoic acid has been titrated, while 150° is reached after octanoic acid has left the column.

This arrangement can, of course, also be used for the analysis of the acids when they are dissolved in the minimum quantity of ether.

Example

The procedure described above was applied to a mixture of fatty acids containing formic acid as

well as acids with a higher molecular weight than valeric acid (Fig. 2).

The separation of the acids, even of formic and acetic acids, proved to be good, while the recovery was as good as that obtained by James & Martin.

SUMMARY

The gas-liquid partition chromatography of volatile fatty acids is modified in such a way that the lower fatty acids, including formic acid, and the higher volatile fatty acids up to dodecanoic acid can be separated and quantitatively determined by one column analysis.

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