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Examination of sugars in cocoa powder by two-dimensional thin-layer chromatography

Recent work on the flavour and aroma of cocoa powder has shown that carbohydrates play an important part in flavour development by combining with amino acids to give volatile carbonyl compounds[1,2]. Accordingly, in order to study the changes that take place in sugars during the various stages of manufacture of cocoa, a two-dimensional thin-layer chromatographic method was employed. By this method the principal sugars present in cocoa powders processed from cocoa beans grown in Ceylon were separated and identified. Boric acid-impregnated silica gel plates were used.

Experimental

Chromatoplates

Clean glass plates (20 × 20 cm) were coated with Silica Gel G (25 g in 50 ml 0.1 *M* boric acid, for five plates) to a thickness of 0.25 mm, using a Desaga Applicator, and dried at room temperature on the aligning tray itself for about 2 h, then used for separation.

Solvents

S1 = *n*-Butanol–pyridine–water (6:4:3)
S2 = *n*-Propanol–ethyl acetate–water (7:1:2)
S3 = Acetone–formic acid–ethanol (3:1:1)
S4 = Ethyl acetate–acetic acid–water (6:3:2)

Spray reagents

(A) 4% Ethanolic aniline, 4% ethanolic diphenylamine, syrupy phosphoric acid (5:5:1). A solution was freshly prepared each day[3].

(B) 4% Ethanolic aniline (5 vols.), 4% ethanolic diphenylamine (5 vols.), syrupy phosphoric acid (1 vol.) and 5 g urea in 80 ml ethanol and 20 ml 2% HCl (2 vols.).

Chromatographic procedure

The sugar extract, prepared by the method of CERBULIS[4], was applied on the Chromatoplate using a self-filling micropipette so as to give a spot containing about 50 μg of sucrose (the major sugar) at 2.5 cm from the two edges. The matrix was broken on the other two edges at 15 cm from the origin. The plate was first allowed to develop by ascending chromatography up to the 15 cm mark in S1 or S2, then dried at room temperature for about 1 h and returned to the second tank, for development in the transverse direction in either S3 or S4. The developed plates were dried at 110° for 15 to 20 min, sprayed with the chromogenic spray reagent A or B and heated at 110° for 10 min. A comparison of the R_F values in the two solvent systems and the different colours obtained with the two spray reagents, A and B, helped to identify the various sugars.

Results and discussion

In contrast to normal procedures it was found unnecessary to activate the plates.

TABLE I

$R_F \times 100$ VALUES OF THE SUGARS OF COCOA POWDER TOGETHER WITH THE COLOURS THEY PRODUCE WITH THE CHROMOGENIC SPRAY REAGENTS A AND B

No. of the spots	*$R_F \times 100$*		*Colours according to Methuen Handbook*[5]		*Provisional identification**
	Solvent S2	*Solvent S4*	*Spray A*	*Spray B*	
1	57	57	9 B 4	20 B 1	Ketose
2	52	52	21 D 3	13 A 4	Aldo-pentose
3	48	37	21 D 3	13 A 4	Aldo-pentose
4	38	33	21 E 2	18 A 5	Glucose
5	20	36	8 B 4	20 B 1	Fructose
6	38	25	16 F 1	21 E 2	Sucrose
7	32	16	21 D 2	18 A 4	Reducing trisaccharides
8	26	18	21 C 2	18 A 5	Melibiose
9	25	13	4 C 1	22 C 2	Raffinose
10	15	07	4 C 1	22 C 2	Stachyose
11	04	18	4 C 1	22 C 2	Unidentified

* Based on colour reaction and R_F.

In fact, activating the plates had the following disadvantages: cracking and peeling off of the matrix when kept inside the tank for development, tenacious attachment of the sugars when activated after the first development, resulting in a low R_F value in the second solvent. It was also more time consuming.

As S1 and S2 move fructose very much less than glucose and sucrose, and S3 and S4 move sucrose less than glucose and fructose, use of S1 and S2 in the first dimension and S3 or S4 in the second dimension made possible the separation of glucose, fructose and sucrose which are the principal sugars in cocoa powder. The above pairs of solvents also help to separate the oligosaccharides, since S_1 and S_2 give high R_F values for them.

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