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Note

Sephadex G-25 thin-layer chromatography

Determination of coloring matter in cane syrup from burned and unburned cane*

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It has been reported[1] that two classes of coloring matter in cane juice and in syrup can be separated on a Sephadex G-25 column. One is a brown mixture of high molecular weight (5000 and above); the other is a mixture containing brown and yellow coloring matter with a low molecular weight (between 150 and 350).

This paper describes the separation of the high- and low-molecular-weight coloring matter in syrup samples from burned and unburned cane obtained from various sugar factories in Hawaii, using the Sephadex G-25 thin-layer chromatographic method reported by Tu and Degnan[2], and the effect of not burning the cane on the syrup color.

EXPERIMENTAL

Five pairs of syrup samples from five factories were used. Each pair consisted of one sample from burned cane and one from unburned cane, both samples from each pair harvested from a single field of identical cane variety and processed under normal operating conditions in the same factory.

The separation and determination of coloring matter in syrup was carried out as described by Tu and Degnan[2]. A 30-g amount of Sephadex G-25 Superfine gel, swelled in excess of 50% (v/v) aqueous ethanol, was thoroughly homogenized and the thick slurry poured into a Desaga/Brinkmann standard applicator (Westbury, N.Y., U.S.A.). The gel was spread over five 20 × 20 cm cleaned glass plates to a thickness of 0.5 mm. Cane syrup containing 60–65% of refractometer solids[3], diluted to about 0.3 g of solids per millilitre, was applied to a prepared plate in three 20-μl spots, 5 mm apart, so that a total of 0.018 g of solids was used. A 5-μl spot of 1% Blue Dextran (Pharmacia Fine Chemicals, Sweden), molecular weight 2 000 000, was used as a reference. The plate was developed horizontally with 50% aqueous ethylene glycol for about 20 min in a Desaga/Brinkmann BN chamber. Duplicate color measurements were made on each of the ten samples.

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Measurement of color

Two large, colored regions were separated on the plate, one being the high-molecular-weight brown region and the other the low-molecular-weight brownish yellow region. Each region was sectioned, removed and transferred into a small test-tube. A 5-ml volume of water was added to the test-tube containing the high-molecular-weight brown portion, while 10 ml of water were added to the test-tube containing the low-molecular-weight matter. The slurries in each tube were stirred and filtered through a Whatman No. 50 paper. The absorbances of the filtrates from each test-tube were determined at a wavelength of 280 nm on a Beckman DK-2 spectrophotometer, as the absorption at this wavelength is reported to be higher than that at any wavelength in the visible region. The attenuation index, a_λ^*, was then calculated[4]. The index a_λ^* is defined in terms of the observed transmittance, T, at a given wavelength, λ, the cell length, b (cm), and the concentration of total solids, c (g/ml), as

$$a_\lambda^* = \frac{-\log T}{bc}$$

The attenuation indices at 280 nm of the high- and low-molecular-weight coloring matter in the syrup from the burned and unburned cane are shown in Table I, which shows that the figures obtained from each duplicate of a sample are close. This suggests that the method can be used in routine determinations for coloring matter of different molecular weights in cane products.

TABLE I

ATTENUATION INDICES (a_λ^*) AT 280 nm OF HIGH- AND LOW-MOLECULAR-WEIGHT COLORING MATTER IN SYRUP SAMPLES FROM BURNED AND UNBURNED CANE

$a_\lambda^* = \frac{A}{bc}$ where A is absorbance, b is path of measuring cell (cm) and c is concentration (g/ml).

Sample from factory No.		*Burned*		*Unburned*	
		High mol. wt.	*Low mol. wt.*	*High mol. wt.*	*Low mol. wt.*
1		29.05	217.10	50.26	229.29
		26.40	217.10	47.79	223.10
	Average	27.73	217.10	49.03	226.20
2		25.09	217.10	51.58	208.43
		22.53	214.19	49.93	205.62
	Average	23.81	215.65	50.76	207.03
3		33.19	194.71	36.88	184.38
		31.09	192.10	35.40	181.90
	Average	32.14	193.41	36.14	183.14
4		33.88	242.19	42.74	249.00
		33.88	235.67	39.70	249.00
	Average	33.88	238.93	41.22	249.00
5		36.02	228.05	36.02	223.10
		34.58	228.05	36.02	223.10
	Average	35.30	228.05	36.02	223.10

Effect of burning on syrup color level

The difference in attenuation index between the syrup samples from the burned and unburned cane is most obvious in the high-molecular-weight fractions. The results indicate that the samples from the unburned cane contained more high-molecular-weight coloring matter than those from the burned cane.

Cane tops and leaves were reported earlier to be the main sources of the high-molecular-weight coloring matter[5]. Assuming a good burn, dried cane leaves should be completely burned, and syrup samples from well burned cane should therefore contain less of the high-molecular-weight coloring matter than those from incompletely burned cane resulting from wet weather prior to cane burning. The lack of any significant difference in sample 5 and the small difference in sample 3 were probably caused by the relatively incomplete burning that took place in these two tests compared with the other tests[5].

As shown in Table I, burning has little or no effect on the level of low-molecular-weight coloring matter because cane leaves are not the only main source of this material[6].

The effect of not burning cane on the quality of the sugar produced was reported in 1970 and 1972 (refs. 7 and 8). The color of the sugar crystal produced from unburned cane is higher than that from burned cane.

REFERENCES

1 C. C. Tu, *Hawaiian Sugar Planters' Association, Experiment Station, Annual Report*, 1971, p. 65.
2 C. C. Tu and M. Degnan, *Int. Sugar J.*, 74 (1972) 259.
3 J. H. Payne, *Sugar Cane Factory Analytical Control*, Elsevier, Amsterdam, 1968, p. 65.
4 C. C. Tu, *13th Congr. Int. Soc. Sugar Cane Tech.*, 1968, p. 416.
5 C. C. Tu, *Hawaiian Sugar Planters' Association, Experiment Station, Annual Report*, 1966, p. 42.
6 C. C. Tu, *Int. Sugar J.*, in press.
7 C. C. Tu, *Hawaii. Sugar Tech. Rep.* (1970) 111.
8 G. E. Sloane and L. J. Rhodes, *Hawaii. Plant. Rec.*, 58 (1972) 173.