

CHROM. 3644

Analysis of the pigments of black tea extracts by chromatography on acetylated Sephadex

Theaflavins, a term incorporating theaflavin itself and theaflavin gallates¹, are responsible for the desirable bright golden appearance of tea liquors² so that methods for measuring the amounts of these pigments are of value in assessing the quality of a tea. Methods which have been used for this determination are based on that of ROBERTS AND SMITH³, in which the theaflavins are extracted from an aqueous infusion by isobutyl methyl ketone, purified to some extent by washing with sodium bicarbonate

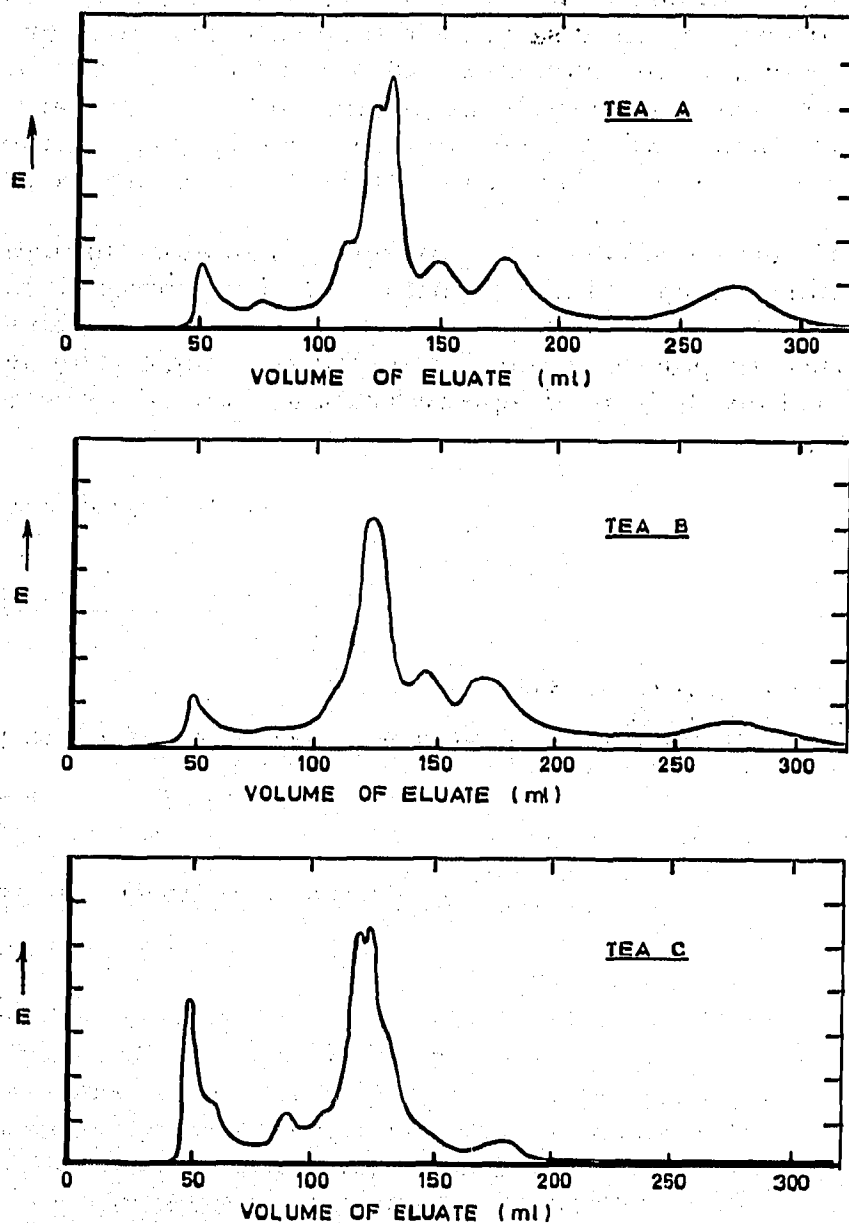


Fig. 1. Elution patterns of three tea extracts.

solution and measured spectrophotometrically. This method is inconvenient and unsuitable for development as an automatic procedure. It has been found that theaflavins can be separated from the other pigments of tea by chromatography on Sephadex LH-20 and this technique is not only suited for automatic operation but is capable of giving additional information on the brown pigments of tea⁴ (the so-called "thearubigin" fraction).

Materials and methods

The Sephadex LH-20 gel (Pharmacia Ltd.) was equilibrated in a Whatman (Reeve Angel & Co., Ltd.) chromatography column (31.5 × 2.54 cm) with 60% aqueous acetone. The tea infusion, prepared under standard conditions, was freeze-dried and a sample portion (50 mg) was suspended in 60% aqueous acetone. Polysaccharide material was removed by centrifugation and the supernatant applied to the column which was eluted with the same solvent mixture. The eluent from the column was monitored at 382 nm by passage through a flow-through cell contained in a Vitatron photometer unit (Fisons Scientific Apparatus Ltd.) connected to a logarithmic-scale recorder.

Results and discussion

Elution diagrams for three different teas are shown in Fig. 1. On the column used in this investigation the elution volume⁵ for theaflavins is 270 ml and it is clear that tea C which is known to be of very poor quality contains no theaflavins. This is in contrast to teas A and B, which are of good and intermediate quality respectively. At present only the theaflavins peak can be interpreted and it is unlikely that absolute values can be calculated for substances under the peak, since different teas contain varying proportions of theaflavin and theaflavin gallates. The remainder of the elution pattern which is different for each tea, illustrates the complexity of the brown colouring matter of tea. Of particular interest is the observation that these coloured materials are probably eluted from the column in order of decreasing molecular size⁴ similar to the behaviour of Crataegus condensed tannins on Sephadex LH-20⁶. The fact that tea C contains a much larger amount of high molecular weight material than tea A confirms BRADFIELD's finding⁷ that good quality teas contain less high molecular weight material than those of inferior quality. As further knowledge of the chemical composition of the various coloured fractions and their contribution to quality becomes available, this technique should be a useful aid to evaluating the characteristics of tea liquors.

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Received June 10th, 1968