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TECHNICAL NOTE

Analysis for caramel colour (Class III)

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Ion-pair high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) methods of analysis have been developed to distinguish Class III caramels from Classes I and IV. These methods show promise for the detection and estimation of Class III caramel added to foods.

INTRODUCTION

There are four classes of caramel colours used as food additives and they are defined by the reactant added to the carbohydrate during the production process (Thornton, 1989). The reactant used in the production of Class III caramels is ammonia whereas sodium hydroxide is primarily used to make Class I caramel and ammonium sulphite to make Class IV. In addition to these reactant possibilities, it is claimed that many variations in colour intensity and subtle flavouring can be produced by varying the conditions of time, temperature and moisture content during caramel production. Thus, for example, Class IV caramels are produced under pressure, but Class III caramels by an 'open pan' process (Myers & Howell, 1992). The consequence of these permutations is that, even within a given caramel class, there can be a wide range of chemical compositions.

Proposed maximum levels of caramel addition to food have been put forward by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1986), in the form of acceptable daily intake levels. Currently there is no widely applicable methodology to determine levels of caramel added to foods.

The aim of this work was therefore to first develop a method for distinguishing Class III caramel from other brown colorants such as food browning reaction products, other classes of caramel and malt extracts. The approach was to identify a fingerprint component common to chromatograms of a wide range of representative Class III samples, but absent from the other colouring materials. The work could then be extended to analysis of foods and the possibility of a semi-quantitative method for Class III detection in foods. The first phase of this work, establishment of a characteristic (fingerprint) component of Class III caramel, is reported here.

EXPERIMENTAL

High performance liquid chromatography

Ion-pair reversed phase HPLC used an ODS 2 column (25 cm × 4·6 mm) and a gradient of 5 mM pentanesulfonic acid in 95:5 water/methanol (pump A), and methanol (pump B). The flow rate was 1 ml/min and the gradient profile was as follows:

Time (minutes)	0	4	20	25	25·1	27	28	29	30	40
% pump B	5	5	70	70	100	100	0	0	5	5

Caramel samples were dissolved in distilled water (5 mg/ml) and injections were 20 μl in volume. Early work to pinpoint possible fingerprint component(s) employed a diode array detector and subsequent work, focusing on the fingerprint component selected, used detection at 275 nm.

Capillary electrophoresis

A SpectraPhoresis 1000 (Spectra Physics) capillary electrophoresis instrument was used, fitted with an open bore 64 cm × 75 μm capillary column. Optimised

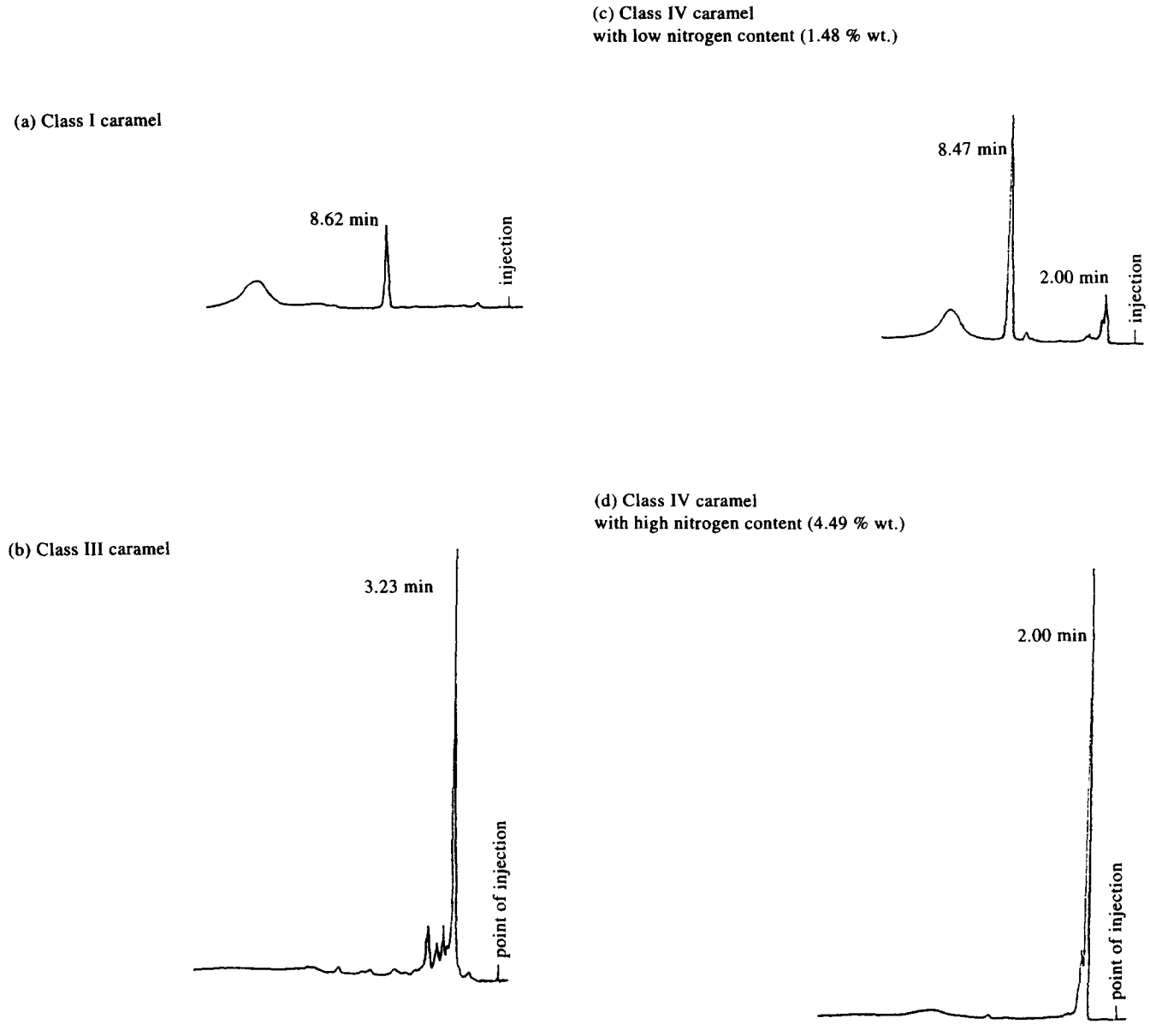

Fig. 1. HPLC traces of caramel colours. (a) Class I caramel; (b) Class III caramel; (c) Class IV caramel with low nitrogen content (1·48% wt); (d) Class IV caramel with high nitrogen content (4·49% wt).

conditions for the separation of the Class III caramels were: 30 mM phosphate buffer (pH 1·9) at 20 kV and 35°C. Caramels were dissolved in distilled water (5 mg/ml) and filtered through a 2-μm syringe filter before analysis. Injections were made in the hydrokinetic mode, loading for 1 s.

RESULTS AND DISCUSSION

HPLC analysis of Class III caramels

HPLC traces of representative examples of caramel Classes I, III and IV are shown in Fig. 1. The fingerprint component selected for Class III analysis appeared as a UV-absorbing peak at around 3·2 min retention time (Fig. 1(b)). This peak was present for all the Class III caramels analysed, comprising a set of 11 samples obtained freshly from manufacturers and a further 13 samples taken from an earlier collection made four years previously. Importantly, the peak was entirely absent from all examples of caramel Classes I and IV run under identical conditions, Figs 1(a), 1(c) and 1(d) for example. Figure 2 shows the relationship between the peak area of the fingerprint component at 275 nm versus the nitrogen content of the fresh caramel samples. A linear relationship between component

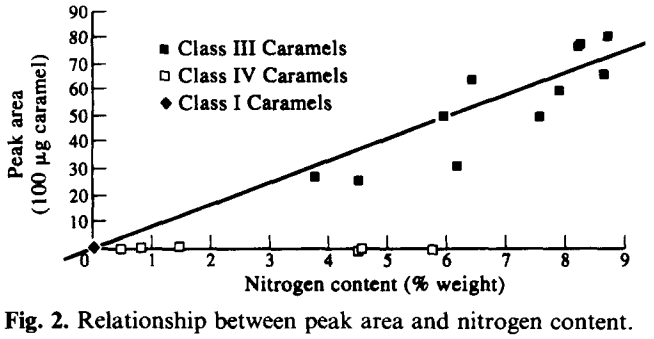

Fig. 2. Relationship between peak area and nitrogen content.

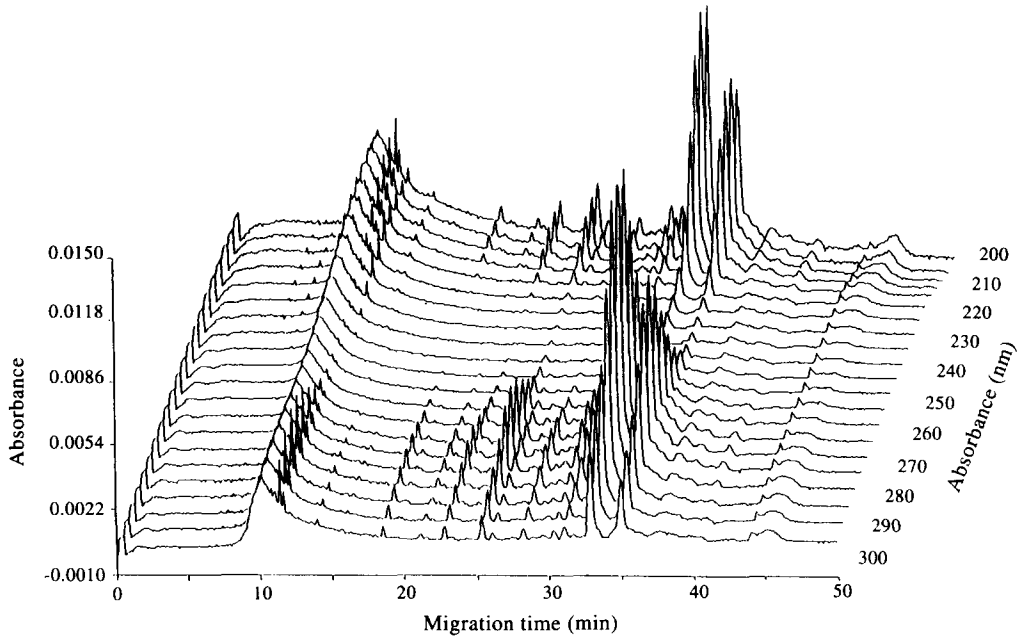

Fig. 3. CE spectrum of a typical Class III caramel.

concentration and the nitrogen content of Class III caramels is evident. Consequently, the rather large heterogeneity of Class III compositions, judged from their nitrogen content, is reflected by the 3·5-fold range in the peak area seen for a given mass of caramel injected (Fig. 2).

Capillary electrophoresis

Distinctive capillary electropherograms have been obtained which can identify the different caramel classes. Two particular peaks stood out in the Class III multi-wavelength electropherogram and Fig. 3 illustrates a representative result. The HPLC component of interest (see above) was responsible for one of the two CE peaks. This was established by collecting the HPLC fingerprint peak and re-analysis by CE (Fig. 4). The HPLC peak was in fact collected for possible NMR and MS analysis in addition to CE analysis. This required the use of an HPLC mobile phase without ion-pair reagent and led to an inferior HPLC separation compared to Fig. 1(b). This could be the origin of the second CE component in the collected fraction, Fig. 4, with a migration time of about 42 min. Quantification of Class III caramel via these CE peaks should be possible although migration times have been found to be somewhat variable, indicating that the CE conditions require further definition or, alternatively, an internal standard should be employed to normalise migration times.

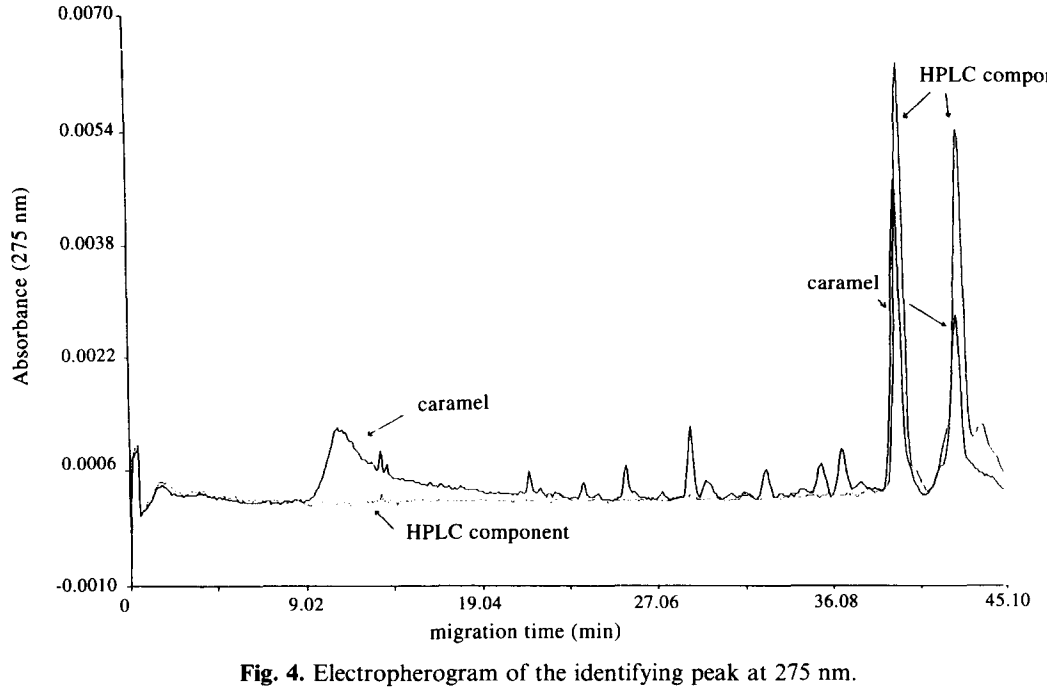

Fig. 4. Electropherogram of the identifying peak at 275 nm.

CONCLUSIONS

Discrimination of Class III caramels from Class I and IV caramels has been achieved using both HPLC and capillary electrophoresis. Analysis by HPLC of the caramels allowed the detection of a peak, which was present in only the Class III samples. The observation of this fingerprint peak in foods could be used to indicate the presence of added Class III caramel and permit a semi-quantitative estimation of the level of caramel in the food analysed. These possibilities are the subject of further investigations.

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