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Use of capillary electrophoresis for monitoring citrus juice composition

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ABSTRACT

New trends in adulteration monitoring, favor the development of methods analyzing simultaneously as many compounds as possible. Capillary electrophoresis has been applied to the examination of a broad spectrum of citrus juice molecules that absorb in the UV and in the visible light. Depending on the conditions up to thirty compounds could be separated. The identified molecules included phenolic amines, amino acids, flavonoids, polyphenols and vitamin C. Samples can be analyzed without specific preparation and the best separations were obtained with diluted solutions due to a stacking effect. This method has been applied to the comparison of pure orange juice and pulpwash, a major adulterant of orange juice. Several significant quantitative differences were seen and it is hoped that this procedure will provide a more precise way of estimating pulpwash in orange juice.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) has become a major separation tool for the rapid analysis of a large variety of molecules [1,2], including amino acids [3], peptides and proteins [2,4], nucleotides [3] and oligonucleodides [5], saccharides [6], oligosaccharides [7], flavonoids [8] or catechols and catecholamines [9]. The ability to separate molecules of a different nature is particularly important when analyzing a natural biological product which may contain hundreds of chemicals. Recently HPCE has been used to investigate biological samples ranging from hepatoma membrane proteins [10] to Maillard reaction products [11].

A large spectrum of compounds with widely different chemical properties have been identified in citrus products [12]. In the past, Petrus and Attaway [13] have estimated various classes of molecules in citrus juices, using UV and visible spectrophotometry and fluorometry. Individual constituents could not be examined since these techniques do not involve a separation process. The analysis of individual citrus components has been achieved by HPLC, a method which requires successive analyses associated with each chemical species [14–17]. Recently, a multiple detector linked with an HPLC system, has been developed by ESA to analyze numerous citrus compounds [18]. HPCE may allow many of these chemicals to be quantitated in a single run. In this study, an HPCE method has been developed that allows the monitoring of citrus products. Compounds present in orange juice, grapefruit juice and pulpwash showed significant quantitative differences.

EXPERIMENTAL

Apparatus

Analyses were performed with a Spectra-Physics 1000 electrophoresis apparatus (Spectra-Physics, San Jose, CA, USA) equipped with high-speed scanning detection in the UV and in the visible for spectral analysis. Separations were performed with uncoated fused silica tubings 70

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cm × 75 μm (J & W Scientific, Folsom, CA, USA) or 70 cm × 50 μm (Polymicro Technologies, Phoenix, AZ, USA).

Sample and buffer preparation

Juice samples were centrifuged for 5 min on a Sorvall RC-5B (DuPont, Newton, CT, USA) centrifuge at 500 *g* to remove any particulate material. Centrifugation was followed by filtration on a polysulfone membrane, dual Acrodisc PF filter (Gelma, Ann Harbor, MI, USA): 0.8-μm prefilter and 0.2-μm filter. In some cases, sodium dodecyl sulfate (SDS) was added to the juice (10 m*M* final concentration) before centrifugation, to enhance the solubilization of the compounds absorbing in the visible. The stacking effect was induced by diluting the juices fourfold with HPLC grade distilled water and injecting the sample for 10 s.

A 50 m*M* solution of phosphate buffer was used as acidic electrolyte (pH 6.8). A 50 m*M* solution of boric acid–borax provided the buffer between pH 7.6 and 9.2. A 50 m*M* solution of borax–NaOH provided the pH 10 electrolyte. Sample were also ran with concentrations of borax (pH 9.3) ranging from 10 to 50 m*M*.

Running conditions

A 100-ppm (w/w) amount of each standard was injected hydrodynamically for 2 s and ran at 18 kV at 30°C, with a solution of 20 m*M* borax pH 9.3 as electrolyte. Originally, single strength juices were injected for 10 s and ran under the same conditions. Amino acids were separated with this buffer using a potential gradient rising from 21 to 25 kV during the first 16 min of the run.

A good separation was obtained with a 10-s injection of diluted juice into a 75 μm column, ran at 30°C with a 35 m*M* solution of borax pH 9.3, under which conditions, the voltage was maintained at 21 kV for the initial 12 min and raised to 25 kV in 30 s for the remainder of the analysis. The optimal conditions were met with a

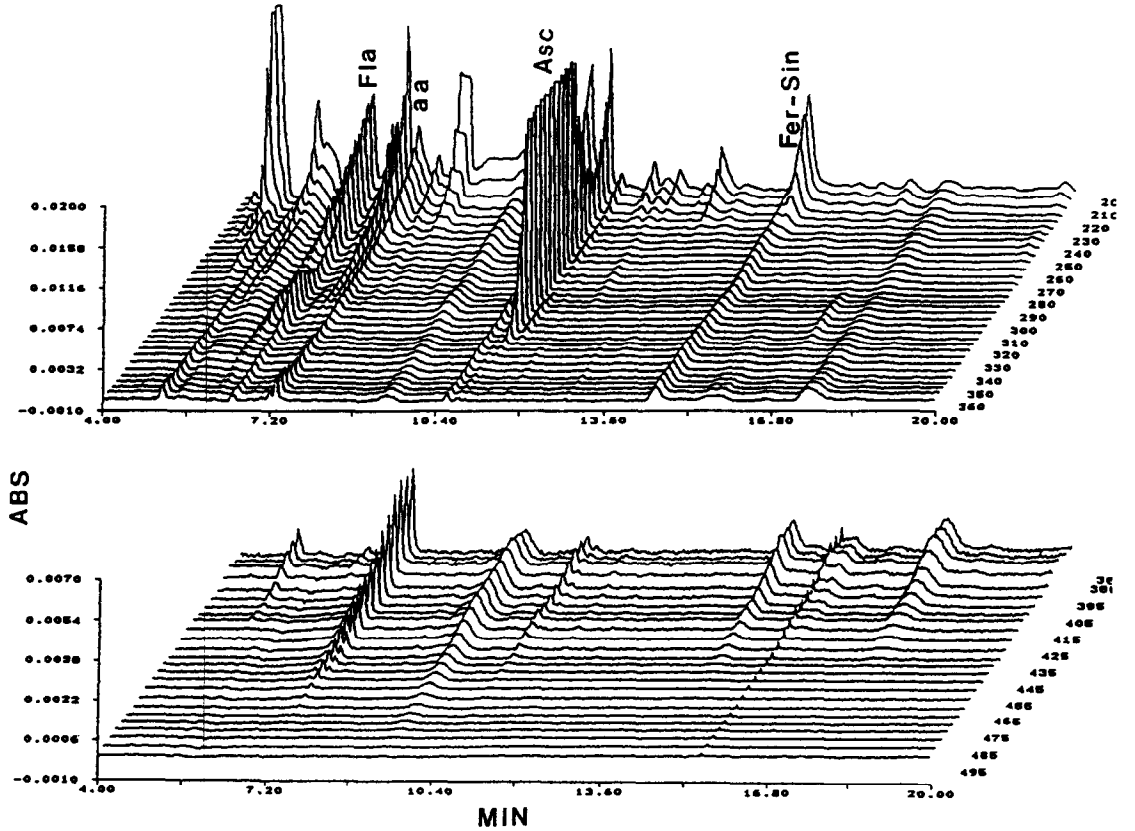

Fig. 1. UV and visible electropherogram of single strength orange juice under the original conditions. Fla = Flavonoids; aa = amino acids; Asc = ascorbic acid; Fer-Sin = feruloyl and sinapyl glucose.

15 s injection into a 50 μm column, at 21 kV and 25°C using the same borax buffer.

RESULTS AND DISCUSSION

The Spectra-Physics system allowed results to be expressed as (1) two-dimensional electropherograms for any given wavelength, (2) three-dimensional graphs containing multiple UV (200–360 nm) or visible (370–500 nm) wavelengths, or (3) spectra of single peaks. The nature of the detectors limited the observable molecules to those absorbing in the UV or the visible light. Therefore, some molecules such as most amino acids, could not be detected with this system. Because of a lack of solubility in aqueous solvents, neutral, hydrophobic compounds such as carotenoids gave little or no

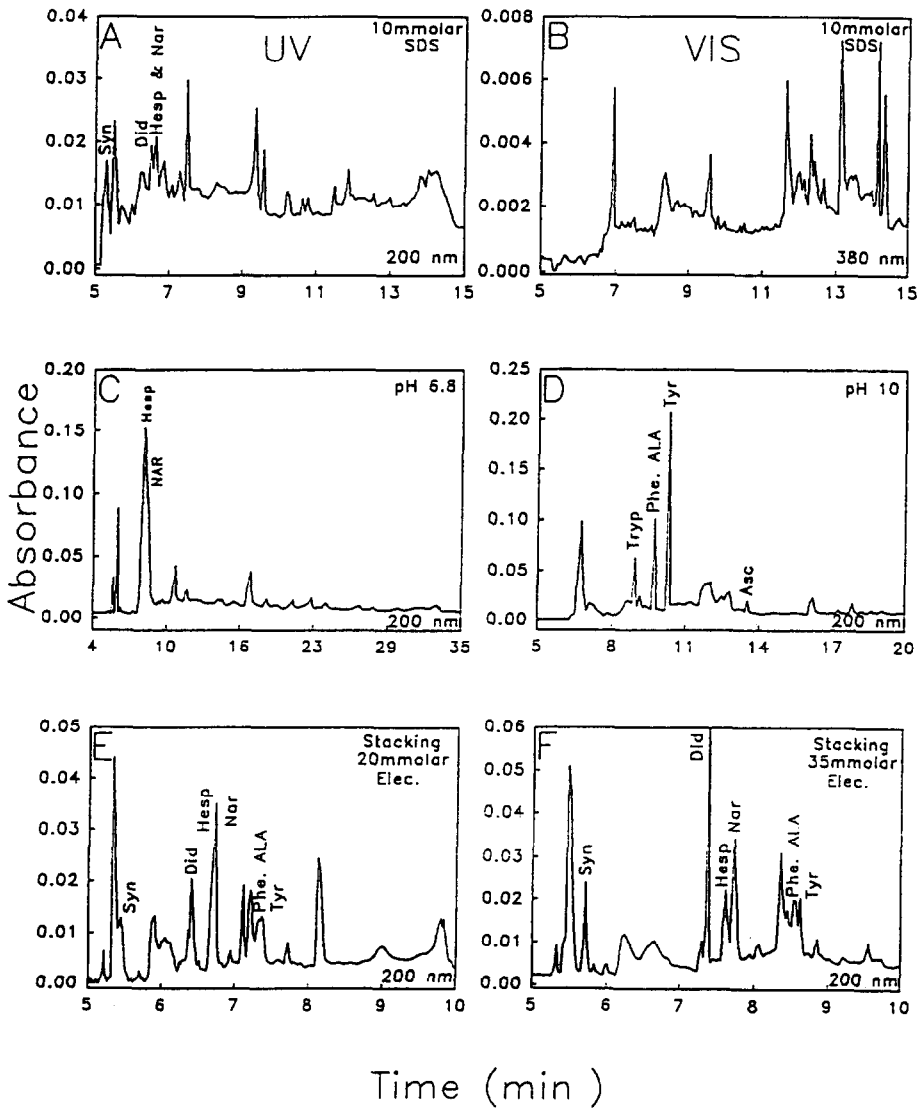

Fig. 2. Effect of the various modifications of the running conditions on the separation of orange juice components. (A) UV and (B) visible electropherograms of orange juice containing 10 m*M* SDS. Electropherogams of juice at (C) pH 6.8 and (D) pH 10 in this case, the juice was spiked with 35 ppm of tryptophan, phenylalanine and tyrosine. (E) Stacking effect at buffer concentration of 20 m*M*, only the first 10 min of a 25-min run are shown, the only major peak not shown is the feruloyl and synapyl glucose peak (see Fig. 5). (F) Electropherogram at the optimal buffer concentration (35 m*M*), also limited to the first 10 min.

response under the present conditions. When concentrated, neutral compounds were seen to move as a single peak displaced by the electro-osmotic flow. Since in orange juice, most of the color is provided by carotenoids, the visible electropherogram of citrus juice products did not show any visible specific peak (Fig. 1). The observed peaks were the continuation in the visible of the spectra absorbing mainly in the UV.

Original conditions

Standards of various molecules present in orange juice were examined under the original conditions. Positive responses were obtained with ascorbic acid, coumarins, phenolic acids, flavonoids, polyphenols, tyrosine, phenylalanine, tryptophan and phenolic amines. The migration time and the spectrum of each standard were used to assess the presence of these molecules in citrus juice electropherograms. Under the original conditions (Fig. 1), amino acids were poorly resolved, hesperidin and narirutin as well as feruloyl and sinapyl glucose were not separated at all. Various modifications of the running conditions were tried in order to improve separation and increase responses.

Optimization of the running conditions

The addition of SDS to the juice sample and to the electrolyte, enhanced significantly the response in the visible portion of the electropherogram but it inhibited the UV response of some molecules, particularly the flavonoids (Fig. 2A and B).

Changes in peak resolution were induced by modifying the pH of the electrolyte. Low pH values (6.8–8.7) resulted in a doubling of the migration time and a major reduction in the response of the slow moving components (Fig. 2C). Above the pK_a of the amine group (pH 10), the amino acids behave as cations and were more easily separated. A very good separation of tryptophan, tyrosine and phenylalanine was obtained at pH 10 with a borate–NaOH buffer using a potential gradient to speed up the analysis (Fig. 2D). However, the procedure provided a somewhat poorer separation of other molecules. The best overall separation for most juice components was obtained at pH 9.3 (Fig. 2E).

Recently, the gel electrophoresis concept of stacking has been applied to CE [19]. In this technique, the sample is prepared in a diluted buffer that has the same composition as the support buffer. This results in the formation of an enhanced electric field at the injection point

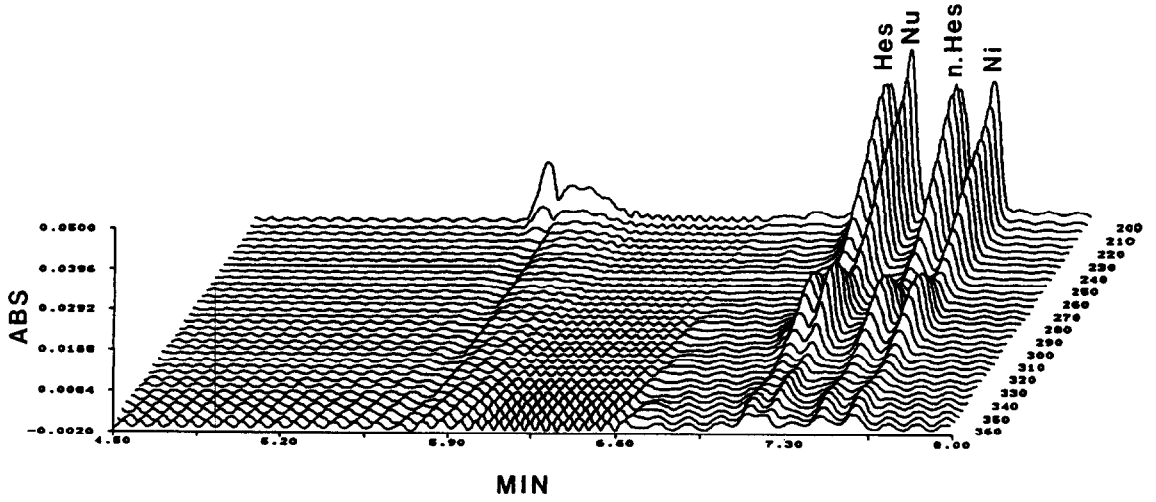

Fig. 3. Electropherogram of the four main flavonoids found in citrus products. Hes = Hesperidin; Nu = narirutin; n-Hes = neohesperidin; Ni = naringin.

and a sharp rise in the number of ions injected into the column. This process is particularly efficient with small ions [19], but since juices contain numerous large molecules, the phenomenon could only be partially reproduced. A significant improvement of the response and of the sharpness of the peaks was, nevertheless, obtained by diluting the juice four-fold in water. The dilution effect was largely compensated by a twelve-fold increase in peak sizes and a much better separation of adjacent peaks (Fig. 2E).

The effect of buffer concentration on separation was also examined. Borax solutions ranging in concentration from 10 to 50 m*M* were used as electrolyte. At low concentration most flavonoids appear as a single peak. Solutions of 50 m*M* provided a relatively good separation of most compounds with a short migration time but highly charged molecules migrated very slowly and therefore gave broad peaks. The best separation of citrus juices was obtained at a borax concentration of 35 m*M* (Fig. 2F). These conditions provided a very good separation for most compounds except feruloyl and sinapyl glucose. Finally, these two chemicals were separated without affecting the results achieved previously

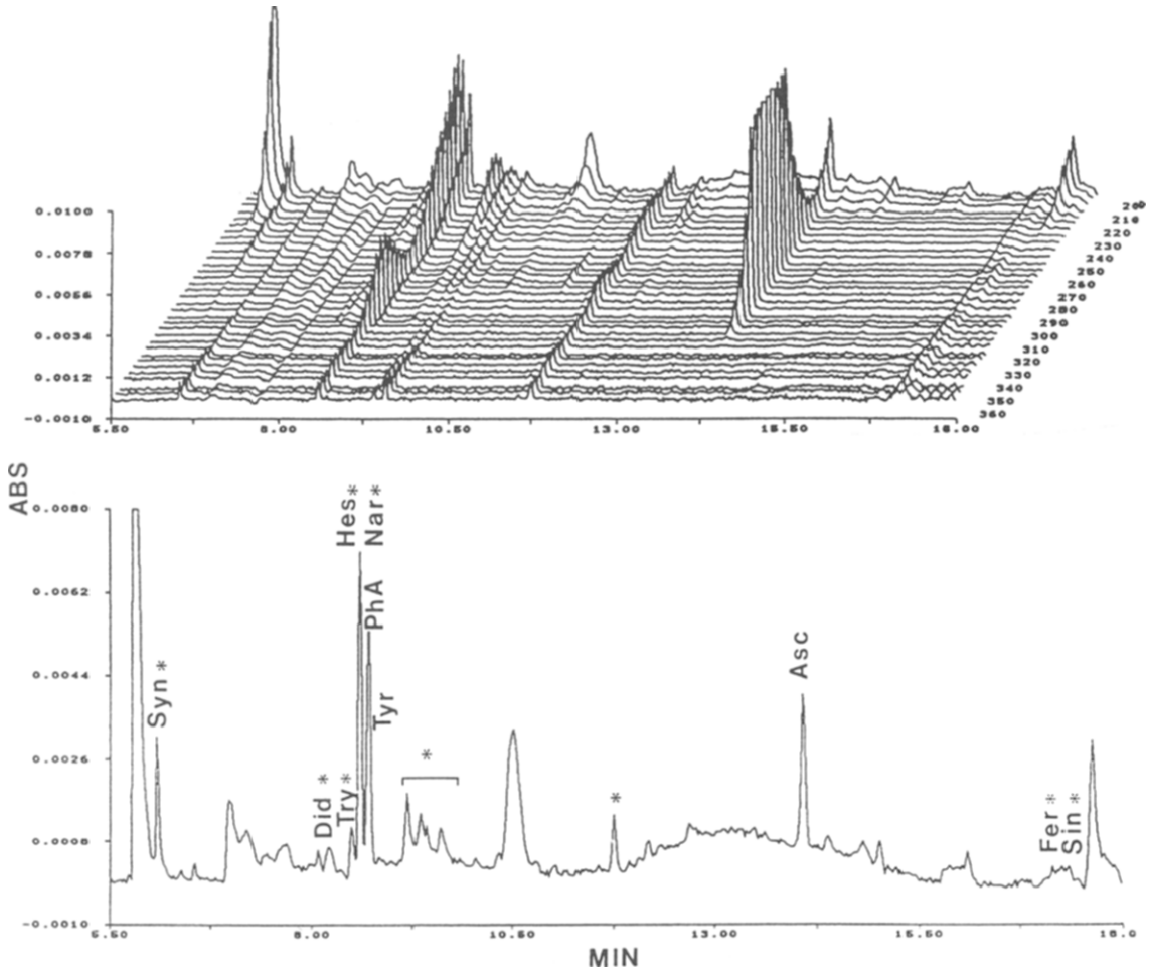

Fig. 4. Multiple and single (200 nm) wavelength electropherograms of orange juice under the optimal conditions. Syn = synephrine; Did = didymin; Try = tryptophan; Hes = hesperidin; Nar = narirutin; PhA = phenylalanine; Tyr = tyrosine; Asc = ascorbic acid; Fer = feruloyl glucose; Sin = sinapyl glucose. The asterisks indicate compounds whose concentrations were always significantly larger in pulpwash.

by using a 50 μm column at 25°C. The identified chemicals separated included: amines (tyramine, synephrine, octopamine), amino acids (tryptophan, tyrosine, phenylalanine), flavonoids (didymin, hesperidin, narirutin, neohesperidin, naringin) (Fig. 3), feruloyl and sinapyl glucose. These latest conditions were adopted for further analyses (Figs. 4 and 5). A very good reproducibility of the migration times was obtained with a relative standard deviation of 0.7% determined for synephrine, benzoic acid and ascorbic acid. Significant changes in retention times were produced only when the column was washed with NaOH.

Juice analysis

This method has been applied to the comparison of citrus products. In grapefruit juices (not shown) the presence of naringin and neohesperidin absent from orange juice could be detected. Analysis of pulpwash, a lower-quality juice product, did not reveal any peaks not already found in orange juice but showed that several components were present in much larger amounts (Figs. 4 and 5). They include synephrine, didymin, tryptophan, narirutin, hesperidin, feruloyl and sinapyl glucose, as well as at least four unidentified molecules, many having coumarin related spectra. These differences are

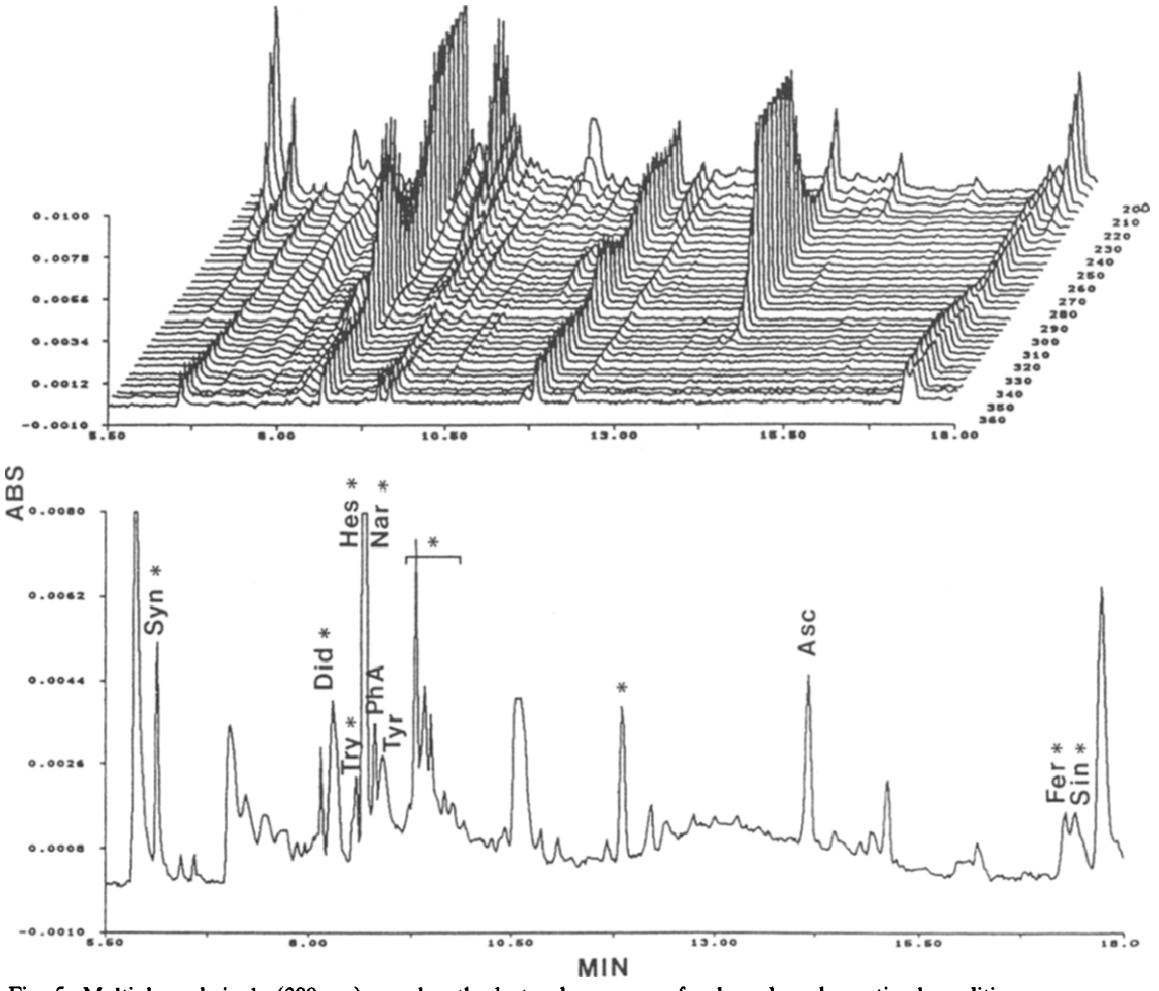

Fig. 5. Multiple and single (200 nm) wavelength electropherograms of pulpwash under optimal conditions.

being used to develop a method for detection and quantitation of pulpwash added to orange juice.

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