Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Grushka, Eli and Kapral, P.(1977) 'Analysis of Capsaicinoids from Various Sources by Gas Chromatography', Separation Science and Technology, 12: 4, 415 — 423
To link to this Article: DOI: 10.1080/00372367708058086
URL: http://dx.doi.org/10.1080/00372367708058086

SEPARATION SCIENCE, 12(4), pp. 415–423, 1977

Analysis of Capsaicinoids from Various Sources by Gas Chromatography

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Abstract

The chromatographic analysis of capsaicinoids and of oleoresin capsicum can indicate relative amounts of the pungent components and the sources of the samples. The present work describes gas chromatographic analyses of several natural and synthetic capsaicins as well as several oleoresins. Use of a high-temperature stationary phase allows good separation of the three major components of oleoresin capsicum. Differences between samples from various sources could be easily seen, both in the general pattern of the chromatograms and in the relative quantities of the major components. Analysis times were relatively short and no chemical derivatization was required.

Capsaicin is one of the more important compounds in the fruits of capsicum. It is present in low concentration in large-sized paprika and in higher concentration in certain chillies. Capsaicin is important in the food industry as a flavoring reagent. In the pharmaceutical area capsaicin is used, alone or in combination with other drugs, as a topical remedy such as an ointment. It is also used in certain cough medicines. Lately, capsaicin has been used as an animal repellent.

Kosuge et al. (*1*) were among the first to realize that natural capsaicin contains more than one component. Bennett and Kirby (*2*), who discussed earlier studies on the composition of capsaicin, indicated that natural capsaicin is a mixture of at least five components: capsaicin (I), dihydrocapsaicin (II), nordihydrocapsaicin (III), homocapsaicin (IV), and homodihydrocapsaicin (V). All these compounds, known as capsaicinoids, are found in extracts of capsaicum; however, the relative amounts of each component vary according to the origin of the fruit. In almost all cases the three most important compounds are capsaicin (I), dihydrocapsaicin (II), and nordihydrocapsaicin (III).

It is of practical importance to be able to determine the major components of capsaicinoids. Such analysis can indicate the source of the capsaicinoids, whether the capsaicin is of natural or synthetic origin, and whether adulterants such as various amides of vanillylamine and fatty acids are present. Although several organoleptic procedures, viz. Scoville (*3*), and spectrophotometric methods (*4–6*) exist, these are unsatisfactory since in most cases they do not distinguish capsaicinoids from some of their cheaper analogs or from compounds having similar functional groups. Chromatography offers the advantages of quick quantitation of the various capsaicinoids in the mixture, and simultaneously the separation from possible contamination. Hence, at least in principle, the origin of the capsaicin can be ascertained. It is, therefore, desirable to develop a simple chromatographic procedure for the analysis of the oleoresin extract of capsicum.

Chromatographic analysis of capsaicin and capsaicinoids has been described by several workers; e.g., Todd and Perun (*7*), Morrison (*8*), Hollo et al. (*9*), Hartman (*10*), Kosuge and Furuta (*11*), Masada et al. (*12*), Quaglio et al. (*13*), Muller-Stock et al. (*14–17*), Todd et al. (*18*), and DiCecco (*19*). While the earlier publications described chromatograms with one or at most two peaks, more recent workers were able to separate several of the capsaicinoids.

The analyses reported previously frequently separated volatile derivatives of the capsaicinoids themselves or of their hydrolyzed fatty acids. In addition, temperature programming was often used to keep retention times relatively short. Most often, however, analysis times were long, mainly because of sample work-up. Consequently, there is still a need for a simple and relatively fast method of analyzing capsicum oleoresin directly. The recent developments of stable high-temperature liquid phases for gas chromatography make it possible to achieve these goals. Previous workers demonstrated that analysis of capsaicinoids can be accomplished. The present paper not only extends the previous studies, but also demonstrates the utility of gas chromatography in determining either the origin or the manufacturer of various oleoresins. This may be of significant importance in the food industry and in various forensic laboratories. Also, applications in quality control are self-evident.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 700 GC equipped with FID was used in this study. Two meter by 0.31 cm (1/8") O.D. stainless steel columns packed with either 10 or 15% w/w Dexsil-300 on 100–120 AW-DMCS treated Chromosorb W were used for the separations. The carrier gas was He.

The oven temperature was set at 240 or 245°C. The injector and detector temperatures were 275°C. The carrier velocity was adjusted to about 16 cm/sec.

Reagents

Natural capsaicin was purchased from Sigma Chemical Co. (St. Louis, Missouri) and from Pfaltz and Bauer Chemical Co. (Flushing, New York). Synthetic capsaicin was obtained from Pfaltz and Bauer Chemical Co. and from K & K Laboratories (Plain View, New York). Samples of oleoresin capsicum containing 3.75% capsaicins, manufactured by Norda Essential Oil and Chemical Co. (New York City) and Kalamazoo Spice Extraction Co. (Kalamazoo, Michigan) were donated by Osmose Wood Preserving Co., Buffalo, New York.

Procedure

Solutions of natural and synthetic capsaicins were made by dissolving a known quantity of the powder in methanol. The concentration range of these standards varied from 0.72 to 10.0% w/w. Depending on the concentration of the standard, anywhere between 1 and 10 μl were injected into the chromatograph with a Glenco or a Hamilton microsyringe.

The oleoresins were injected directly into the chromatograph. Initially, extraction of the capsaicinoids with methanol was tried. However, it appeared that the extractions were not complete and eventually this procedure was stopped.

RESULTS

The first aim of the present study was to examine the chromatographic difference between synthetic and natural capsaicin. In a typical chromatogram of synthetic capsaicin, as might be expected, there is only one prominent peak. Synthetic capsaicin from various vendors gave virtually the same chromatograms. Figure 1 shows a chromatogram of natural capsaicin obtained from Pfaltz and Bauer (2.0 μl of 1.0% w/w solution). With the exception of the impurities eluting in the first 5 min of the run, three prominent peaks are apparent. These were assigned as capsaicin (I), dihydrocapsaicin (II), and nordihydrocapsaicin (III). The identification was based on the chromatogram of synthetic capsaicin, the relative amounts of the three components, and on the order of the elution. The

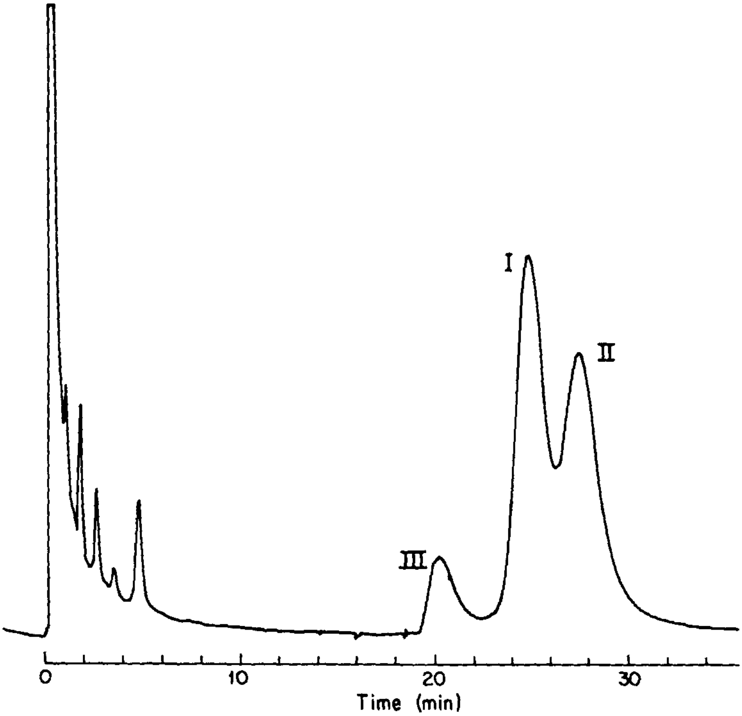

FIG. 1. Chromatogram of natural capsaicin. Source: Pfaltz and Bauer. 15% Dexsil-300 stationary phase. Column temperature: 240°C.

McReynolds constants of Dexsil-300 (*20*) indicate that this phase is only slightly more polar than SE-30, which was used by Masada et al. (*12*) and by Muller-Stock et al. (*14–17*). These workers assigned retention orders using mass spectrometry. The retention order given here is based on the similar characteristics of SE-30 and Dexsil-300. No peaks corresponding to homocapsaicin or homodihydrocapsaicin could be observed. Since no standards of these two compounds were made (to our knowledge, no chemical company sells the pure compounds), it could not be determined whether the present natural capsaicin did not contain them to any appreciable amounts or their elution time coincided with those of peaks I, II, and III. Judging from reports of Masada et al. (*12*) and Muller-Stock et al. (*14–17*), however, homodihydrocapsaicin, if present to any appreciable extent, should elute after dihydrocapsaicin. In any event, it should be realized that these two compounds are only minor constituents of capsaicinoids. The resolution shown in Fig. 1 is very good. Better resolution was observed at 220°C with a 10% Dexsil column, but the analysis time was increased to about 35 min. By increasing the temperature to 235°C the analysis time was decreased to 22 min while still maintaining very good peak separations.

Figure 2 shows a chromatogram of natural capsaicin, obtained from Sigma Chemical Co., under the same condition as shown in Fig. 1. The amount injected was 3 μl of 0.72% w/w solution. It is apparent that either the source or the method of obtaining the capsaicinoids is different in the two cases. The early impurity peaks are missing in the Sigma sample, and the relative amounts of capsaicin to dihydrocapsaicin appear to be different. In the Pfaltz and Bauer sample the relative amounts of the three components were: I, 48.9%; II, 43.4%; and III, 7.64%. In the Sigma sample the relative amounts were 47.8, 46.7, and 5.74%, respectively. The measurements were made only in cases where the resolution was good enough to minimize the error of estimating the peak area. Areas were obtained by multiplying the peak height by the width at half the height. It was also assumed that the three peaks constituted the total amount of capsaicinoids, and that the detector response was the same for all components. Hence the numbers given should be taken only as "order of magnitude" values. Although the number of experiments is not large enough to allow statistical studies, it seems that, indeed, the composition of the so-called natural capsaicin is different in the samples from the two sources.

The above natural samples of capsaicinoids were most likely extracted from oleoresin capsicum. Figure 3 shows a typical chromatogram obtained from injecting 1 μl of oleoresin from Kalamazoo Spite Extracting Co.

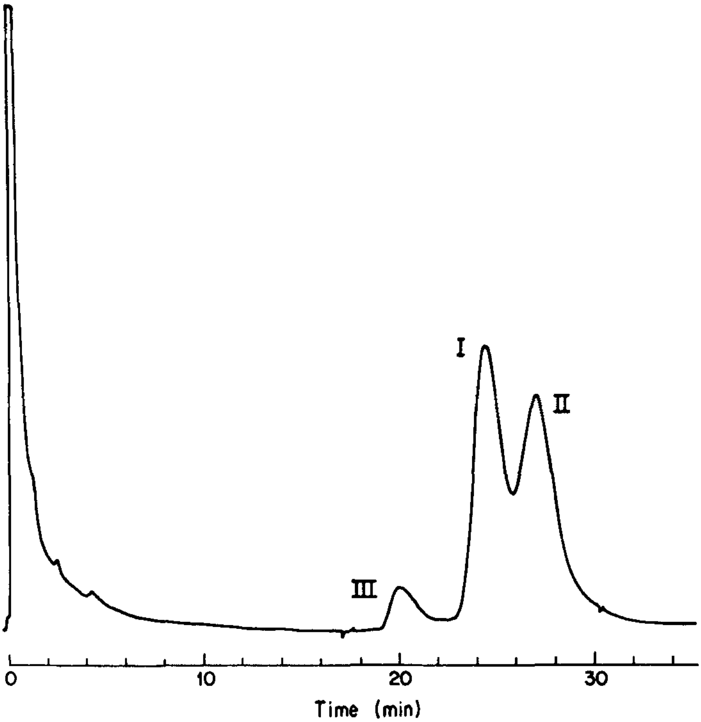

FIG. 2. Chromatogram of natural capsaicin. Source: Sigma. Same condition as Fig. 1.

Capsaicin (Peak I), dihydrocapsaicin (Peak II), and nordihydrocapsaicin (Peak III) are observed, in addition to other unknown peaks. Homodihydrocapsaicin, indicated as Peak IV, might also be present. However, conformation of the validity of this assignment is uncertain at this stage. Figure 4 shows a typical chromatogram of 1.0 μl of oleoresin obtained from Norda. It seems that the Norda sample contains slightly less dihydrocapsaicin and nordihydrocapsaicin. However, there are some unknown compounds which elute after the identifiable capsaicinoids. The chromatograms indicate that the quantities of these unknowns are quite large. The elution pattern before the emergence of capsaicinoids is also different in the two samples. It should be noted that according to the manufacturers, both oleoresins contained 3.75% capsaicinoids. This was verified by Kapral (*21*) using a modified extraction and spectrophotometric analysis

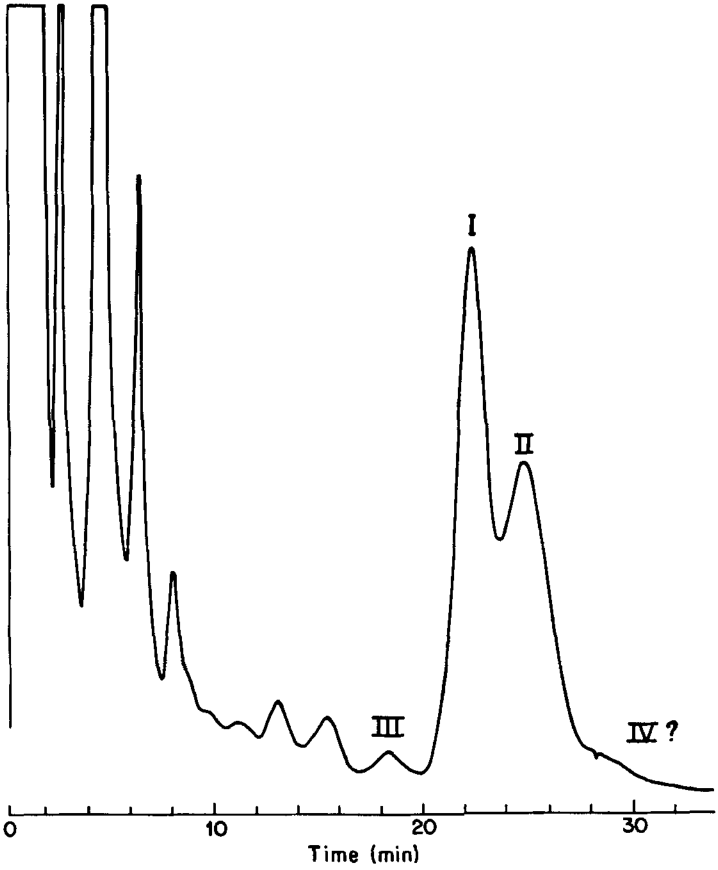

FIG. 3. Chromatogram of oleoresin capsicum. Source: Kalamazoo Spice Extracting Co. 10% Dexsil-100. Column temperature: 245°C.

method. Yet, the chromatograms show obvious differences between the two samples. These results again indicate the unsatisfactory nature of the UV absorption tests.

By lowering the temperature to 215°C, an approximate quantitation of Peaks I, II, and III in the Kalamazoo sample could be made. The relative composition was about 50.3% of I, 42% of II, and 7.7% of III. It must be stressed that these are approximate values only. The nature of the complex elution pattern makes it very difficult to establish the position of the baseline. If accurate quantitation is needed, the standard addition

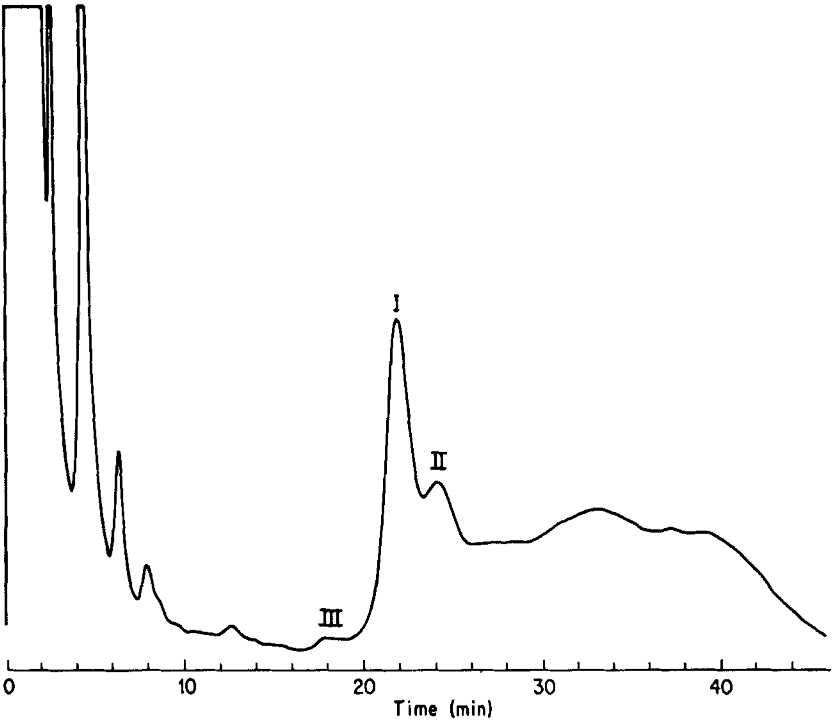

FIG. 4. Chromatogram of oleoresin capsicum. Source: Norda Co. Same condition as Fig. 3.

method should be used. In the case of the Norda sample, due to the unknown solutes eluting after Peak II, even an approximate quantitation could not be made. Nonetheless, it is clear from the chromatograms that, as was mentioned before, the ratio of Compounds I to II in the Norda sample is different from that in Kalamazoo's.

The analyses reported above were made using samples from the same lot of each oleoresin. At this stage variations from lot to lot, coming from the same supplier, are unknown; however, this point is worthy of further investigation.

Preliminary tests with a Japanese oleoresin from Iwatani Co., containing 4.5% capsaicinoids (according to the manufacturer, and tests made at Osmose Co.), gave a chromatogram which was entirely different from the Kalamazoo and the Norda samples.

Acknowledgments

The authors thank Osmose Wood Preserving Co., Buffalo, New York, for the generous supply of various oleoresins. This work was supported in part by a grant from the Student Association of the State University of New York at Buffalo.

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Received by editor November 8, 1976