be used, while still meeting the discussed CAOBISCO requirement of 5.0 ± 2.0% CBE in the chocolate.

Calculation of the CBE content using the extreme cocoa butter C_{52} values and the corresponding C_{52} values for the CBE band intercepts expresses the results as percentages including the maximum possible errors. For routine analysis, the calculation may be simplified by using only the mean C_{52} cocoa butter value and the corresponding intercept values on a line running through the center of the CBE band. For the example shown in Figure 2, for instance, values of 15.7% in the fat phase or 4.7% in the chocolate would result. If values greatly exceed 5.0% in the chocolate, the entire calculation procedure must be completed to determine the maximum possible error.

ACKNOWLEDGMENTS

C. Bishop determined the triglyceride analysis and B. Stubbs prepared the chocolate samples.

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APPENDIX

Definition

The following definition of vegetable fats has been drawn up for the purposes of the EEC directive: edible vegetable oils and fats are lipids obtained from vegetables, the predominant glycerides being triglycerides.

They may contain small amounts of other components of lipids such as mono- and diglycerides, polar lipids, free fatty acids and unsaponifiable matter.

They may be fractionated, hydrogenated, inter- or intraesterified and/or refined.

Analytical Criteria

The vegetable fats for use in chocolate within the Community must comply with the following analytical criteria in order to allow qualitative and quantitative control:

(a) Level of triglycerides type SOS ⩾ 65%.
(b) Fractions of the 2-position of triglycerides, occupied by unsaturated fatty acids, ⩾ 85%.
(c) Total content of unsaturated fatty acids, ⩽ 45%.
(d) Unsaturated fatty acids with 2 or more double bonds, ⩽ 5% (this figure is included in [c]).
(e) Level of lauric acid, ⩽ 1%.
(f) Level of *trans* fatty acids, ⩽ 2%.

Work is continuing on certain of these analytical criteria and if this leads to any proposed changes in the figures now indicated, the EC Commission will be informed.

Level of Use and Declaration

The use of added vegetable fats should be limited to 5% of the total weight of chocolate in the product. The presence of added vegetable fats should be indicated in the list of ingredients. (CAOBISCO has already accepted that a full declaration of ingredients should be applied to chocolate products, in accordance with the provisions of the labelling directive.)

Gas Chromatographic Separation of Long-Chain Fatty Nitriles and Long-Chain Acid Amides

C.N. WANG and **L.D. METCALFE**, Akzo Chemie America*, Research Laboratories,
8401 W. 47th Street, McCook, IL 60525

ABSTRACT

A method is described for the chromatographic separation of long-chain fatty nitriles and long-chain acid amides on a cyanopropyl silicone column. We found that better separations were obtained for these compounds using a cyano column than with any column previously reported.

INTRODUCTION

The long-chain nitriles are neutral materials and are easily chromatographed on many types of columns. Apiezon, silicone type and Carbowax 4000 monostearate have been reported to give excellent separations (1,2). If the separation of saturates from unsaturates and polyunsaturates is desired, a polyester column can be used (3-5). The nitrile group is highly electronegative, probably the most polar of all functional groups, and this polarity becomes apparent when a separation is made on polar and nonpolar type columns. On a DEGS column, the retention time for a nitrile of a given chain length is almost double that for a methyl ester of equal chain length. From this observation, one would predict that liquid phases containing nitriles would be useful, highly polar substrates. This observation has been verified by Tenny and others (6-8).

In order to obtain rapid separations of nitriles without losing the valuable saturate-unsaturate separation, phosphoric acid treated polyesters are useful (4). However, the limited resolution of saturates and unsaturates, as well as the retention time of this column, does not offer the optimum condition for nitrile separations. The described cyanopropylsilicone column, as predicted earlier, will give superior separation of fatty nitriles by gas chromatography.

Long-chain acid amides derived from fatty acids are high-melting, waxlike substances. They are used in many commercial applications. These materials have a very low degree of volatility. One would not generally consider the amides as suitable samples for gas chromatographic analysis. Considerable research effort has been made to chromatograph these compounds. The amides have been separated on Apiezon L-KOH columns (9). Amides have also been resolved on the Versamid 900 column (10). The trifluoroacetyl derivatives of fatty amides have been made and separated (11). Short-chain amides have been resolved on the Dowfax 9N9 column (12). When unsubstituted amides are chromatographed on a DEGS (13,14) and a polyester phosphoric acid column, the peaks that emerge are reported to be nitriles resulting from on-column dehydration of the

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*Formerly Armak Company.

amides. The most popular separation of amides is done on silicone columns (15) such as Dexsil, or by converting them to methyl esters by refluxing them in a methanol HCl solution (10). The resulting methyl ester can be analyzed by using any polyester column. Amides of C10-C24 carbons can be chromatographed on cyanopropyl silicone columns. The separation of chain lengths and saturated from unsaturated amides can be achieved in ca. 25 min.

EXPERIMENTAL

Instruments

A Varian 3700 gas chromatograph (GC) with a CDS-111C automatic integrating system was employed for this work. This instrument was equipped with dual flame-ionization detectors.

The best results were obtained with 3-ft columns of ⅛" stainless-steel tubing packed with a 5% mixed cyanopropyl silicone liquid phase (2-parts Silar 5-CP and 3-parts Silar 7-CP) on Chromosorb W AW DMCS treated 100-120 mesh. Silar is a registered trademark of Silar Labs (16,17). Equivalent products can be obtained from Supelco Inc., Bellefonte, PA, under the trade names SP 2300 (Silar 5CP) and SP 2310 (Silar 7CP).

Instrument Conditions

Nitrogen was used as the carrier gas at the flow rate of 25 mL/min. The column oven temperature was always kept between 180-240 C, depending on the compounds to be separated. The injector temperature was 280 C, and the flame-ionization detector temperature was 290 C.

Samples and Sample Preparations

The nitriles and amides used were made from commercial coco, tallow or erucic fatty acids. No special sample preparation was required. The samples were dissolved in a suitable solvent to obtain a 0.2-0.5% solution before being injected into the GC. Aliquots of 1 μL were usually injected.

RESULTS AND DISCUSSION

The separation of nitriles on the basis of chain-length distribution was not a problem. This can be done on almost any silicone liquid phase column. However, when more detailed information is required, such as the separation of both saturated and unsaturated chains, getting rapid and complete resolution on polyester phase columns is difficult. Cyanopropyl silicone phases were described as early as 1962 by Rotzsche (18,19). Since then, only limited amounts of work have been published on them up to 1972 (20). However, no one has described the use of cyanopropyl silicone liquid phases for separating either long-chain nitriles or amides. Because of the nature of the fatty nitrile and amides, a very polar liquid phase should be used to achieve the optimum separation. The cyanopropyl silicone phase meets this requirement. In Figure 1 is a chromatogram of the separation of commercial coco nitrile—a complete resolution of saturates and unsaturates is observed. The separation is done on a 3-ft mixed Silar phase column. The order of separation is similar to that which would be obtained with methyl esters on a polyester column. The saturates elute before the unsaturates of the same carbon number. In order to examine the separating power of cyanopropyl silicone phase column on nitriles, a commercial erucic nitrile was analyzed. A complete separation of the components was achieved in 3 min, as shown in Figure 2.

The separation of long-chain alkyl amides by GC has always been difficult. In most cases, the hydrolysis method followed by converting the resulting fatty acids to methyl esters has been used to determine chain-length distribution. However, the use of cyanopropyl silicone phase columns makes the GC analysis of fatty amides much easier. Figure 3 shows a chromatogram of a commercial coco amide on a 3-ft cyanopropyl silicone phase column. The order of separation is similar to that which would be obtained with methyl esters on a polyester phase column. The saturated

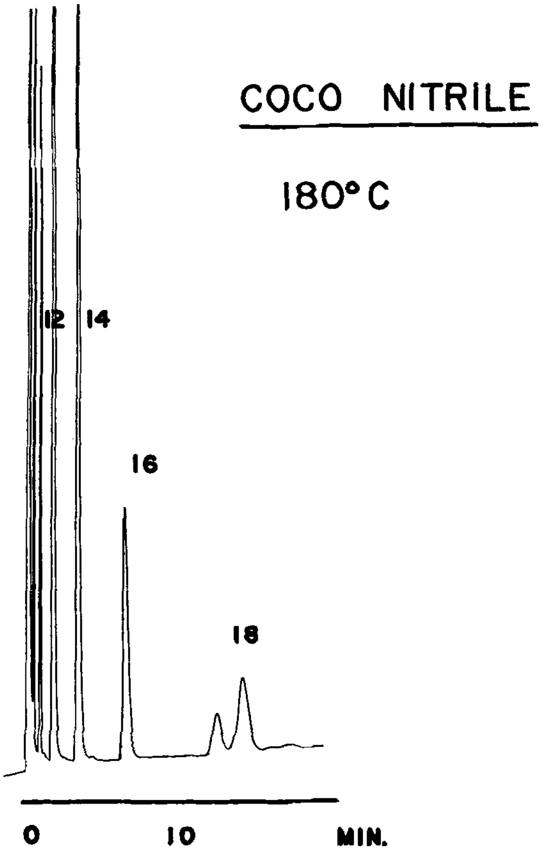

FIG. 1. A commercial coco nitrile chromatographed on a 3-ft Silar mixed phase column at 180 C.

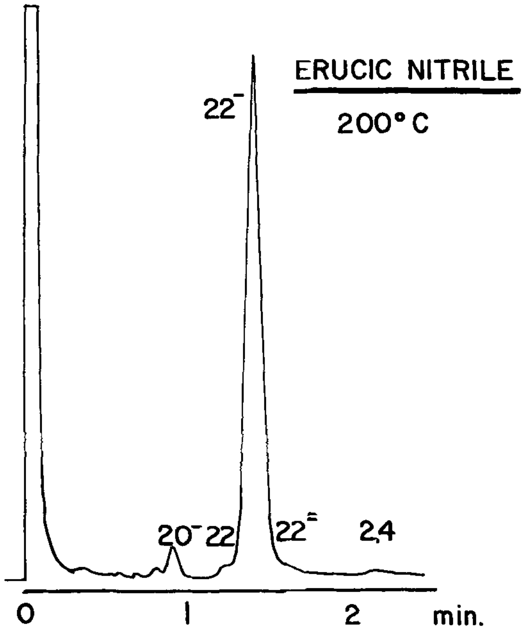

FIG. 2. A commercial erucic nitrile chromatographed on a 3-ft Silar mixed phase column at 200 C.

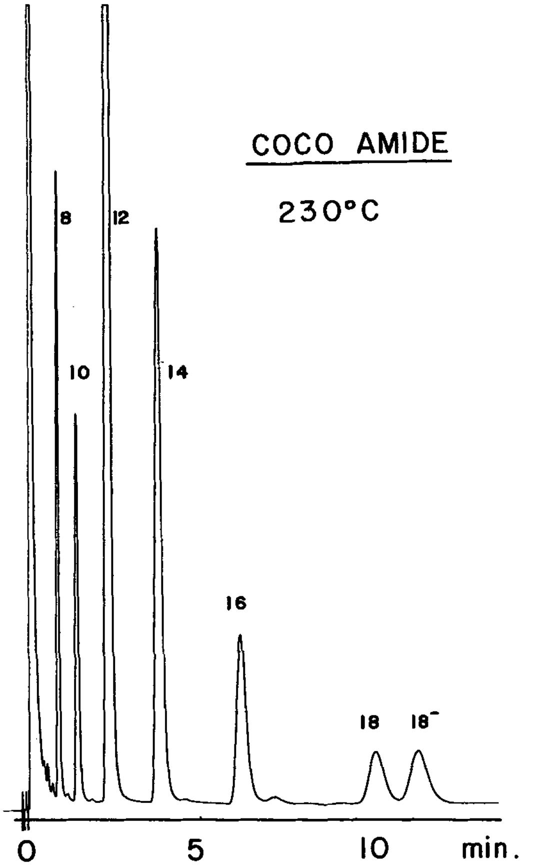

FIG. 3. A commercial coco amide chromatographed on a 3-ft Silar mixed phase column at 220 C.

amides elute before the unsaturated amides of the same carbon number.

Figure 4 shows the chromatogram of a commercial erucamide on a 3-ft cyanopropyl silicone phase column. The column separates not only the amides but also the impurities such as methyl esters and nitriles.

We found that certain details must be followed when using the cyanosilicone columns. No alkaline materials should come in contact with the cyanopropyl silicone phase column. The alkaline substances are detrimental to this liquid phase (21). Isothermal column temperatures should be used to obtain optimum separation. The cyano liquid phases should be used only on acid washed, DMCS treated supports.

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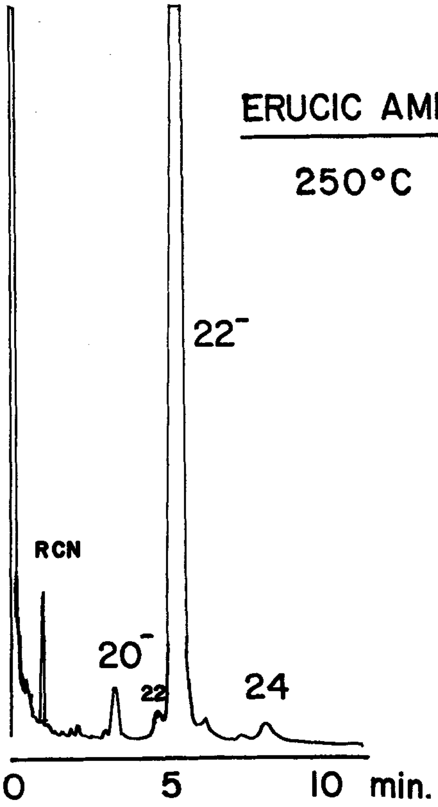

FIG. 4. A commercial erucic amide chromatographed on a 3-ft Silar mixed phase column at 240 C.

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