FLAVOR ANALYSIS BY GAS CHROMATOGRAPHY: (A) GLASS CAPILLARY COLUMNS WITH POLAR PHASES AND SILANOX; (B) MICROREACTOR FOR SUBTRACTION CHROMATOGRAPHY; (C) ODOROUS COMPOUNDS FROM HEATED RAPESEED OIL.

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BY

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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This work is dedicated to my wife and to my mother.

ABSTRACT

McKeag, Richard Gordon. Ph.D., The University of Manitoba, October, 1977. Flavor Analysis by Gas Chromatography: (A) Glass Capillary Columns with Polar Phases and Silanox; (B) Microreactor for Subtraction Chromatography; (C) Odorous Compounds from Heated Rapeseed Oil.

Major Professor; F.W. Hougen

The quality of heated rapeseed oil has often been criticized because of undesirable odors. The discontinuance of the use of rapeseed oil among consumers has been linked to the unpleasant odor upon heating. Little information has been published concerning the chemical components responsible for heated rapeseed oil odor. Such information would be of use to researchers studying rapeseed oil quality, and to industrial processors concerned with quality control and the elimination of off-flavors.

To fractionate chemically complex odor concentrates obtained from heated rapeseed oil by gas chromatography, a conventional gas chromatograph was modified to product an all-glass system for splitless injection to capillary columns. Extruded glass capillaries were coated by a novel technique to produce polar phase support coated open tubular columns with Silanox. A chloroform-acetone solvent combination was used with a two-step dynamic coating procedure.

Columns were prepared with Silanox and the liquid phases OV-225, OV-210, OV-101, Silar-5CP, and Carbowax 20M TPA. Effective plate heights of about 1.0 mm were obtained with large bore columns with lengths up to 135 m. Columns exhibited high thermostability, durability, and sample capacity, tolerating splitless injection of 0.1-0.3 µl

samples for periods of more than 6 months before appreciable losses in efficiency occurred.

A simple microreactor was designed and fabricated for subtraction gas chromatography, an identification technique for chromatographic peaks. The microreactor, inserted in the heated injector port of a gas chromatograph, may be used at an optimum temperature for a reagent independent of the analytical column temperature. Its use was demonstrated for the quantitative subtraction of alcohols by boric acid, aldehydes with o-dianisidine, and aldehydes plus ketones with benzidine, from simple test mixtures. With this microreactor, sodium bisulfite was found to be an inefficient reagent for the subtraction of carbonyls.

An odor isolate collected from rapeseed oil heated to 185°C for 2.5 hr represented 0.054% by weight of the original oil. Gas chromatographic analysis revealed that all five oil samples tested contained the same 138 components. The 15 largest components constituted 86% of the odor concentrate.

Organoleptic evaluation of the chromatographic peaks from the oil odor concentrate revealed a number of "oily" and "rancid" components which presumably are the major contributors to the off-odors of the heated oils.

Twenty-two compounds were identified from the odor concentrate by gas chromatography-mass spectrometry. These compounds included C_5 - C_{10} saturated *n*-aldehydes; *trans*-2-hexenal; 2,4-heptadienal; 2,4-decadienal (two isomers); C_6,C_8,C_9 saturated *n*-acids; 2-heptanone; 2-octanone; and ethyl hexanoate. In addition, propyl propionate and

2-hexanone were identified by gas chromatographic retention time only. Two additional compounds were established by mass spectrometry to be hydrocarbons but their structures were not fully elucidated. The 26 identified compounds and the 11 aldehydes represented 54% and 29.6% of the odor concentrate, respectively.

An assessment of the odor contribution of a commercial antioxidant mixture showed that antioxidants did not contribute to the odor of the heated oil.

The five commercial rapeseed oils showed a slight degree of oxidation prior to heating, as measured by hydroperoxide and thiobarbituric acid values. Heating of the oils with air purging produced sufficient degradation of the oils to be detected as off-odors but not sufficient to be detected as changes in the fatty acid composition.

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GENERAL INTRODUCTION

The 1976-77 world production of edible oil obtained from rapeseed has been estimated by Mielke (1976) to be 2.605 million metric tons. At this production level, rapeseed oil ranks 6th largest among vegetable oils. The success of rapeseed as an economically viable crop in Canada is in part due to successful plant breeding programs; namely, the reduction of glucosinolates in the meal and erucic acid in the oil. The major edible products obtained from rapeseed oil are margarine, shortening, and salad and cooking oils.

Fried and deep fat fried foods are popular items in our diet. A major reason for the popularity of these foods is the characteristic flavor produced during the frying process. Under these conditions, volatile and non-volatile decomposition products of the oil are produced which may have disagreeable odors or affect the quality of the food.

The flavor- and oxidative stability of rapeseed oil has often been criticized. Sulphurous and rancid odors have been attributed by Moser *et al.* (1965) to isothiocyanates and degradation products of linolenic acid, respectively. A recent study by Dobbs and Vaisey (1975) has shown that rapeseed oil heated to frying temperatures emitted significantly stronger unpleasant odors than other popular cooking oils. Discontinuance of the use of rapeseed oil among consumers was linked to unpleasant odor upon heating.

Although much is known about the mechanisms of degradation of triglycerides and fatty acids, and about the volatile decomposition products of some edible oils, there is little information available concerning compounds responsible for the odor of heated rapeseed oil. Such information would be of interest to researchers studying rapeseed oil quality, and to industrial processors concerned with quality control and the elimination of off-flavors or their precursors.

The basic goal of the flavor chemist is the objective characterization of flavor through comprehensive chemical analysis — a formidable challenge for the analyst. Although it is difficult to generalize, odors, the major component of flavors, usually share certain common characteristics, regardless of their origin.

1. The isolation and concentration of odors is difficult because they occur in extremely low concentrations in foods. Buttery *et al.* (1971) estimated that the odor concentrate obtained from tomato was of the order of 10 ppm of the whole fruit. Odorous compounds isolated from chicken by Nonaka *et al.* (1967) represented about 20 ppm of the meat.

2. Food odors are usually complex multicomponent mixtures. Gianturco *et al.* (1974) detected 301 volatile constituents from black tea. Wick *et al.* (1969) reported the presence of about 200 volatile constituents from banana..

3. Trace components in volatile concentrates may have major effects upon the odor. Meilgaard (1975) reported that the odor thresholds of 239 aroma volatiles varied over a concentration range of $2x10^{12}$.

4. Odor compounds are often labile substances which are easily rearranged, degraded, or lost through evaporation or adsorption. In his review on the status of flavor chemistry, Sundt (1970) notes that *cis*-3-hexenal is a sensitive compound easily rearranged into *trans*-2-hexenal. *Cis*-3-hexenal has a fresh green leaf odor with a threshold value 30 times lower than *trans*-2-hexenal which has an astringent bitter green leaf odor.

In view of these and other examples, it is evident that only highly efficient separation techniques, combined with sensitive detection, can offer any possibility of revealing the composition of an aroma.

The major objectives of this study were to develop a simple, direct method of isolation of an aroma concentrate from heated rapeseed oil and to separate the aroma concentrate into its components for organoleptic evaluation and chemical identification.

For clarity, this thesis has been divided into three sections, each containing an introduction, a review of pertinent literature, a description of materials and methodology, a discussion of preliminary experiments and final results obtained, and a summary and conclusions. References are cited in the text using the author-and-year system with a general bibliography at the end of the thesis listing authors alphabetically.

The first section describes the manufacture and use of highly efficient glass support coated open tubular columns for gas chromatographic separations and the conversion of a conventional gas chromatograph to an all-glass capillary system.

The second section describes the design and use of a microreactor

for subtraction gas chromatography, an ancillary technique used for the identification of chromatographic peaks. This technique eventually was not used for rapeseed odor component identification as a sensitive gas chromatograph-mass spectrometer became available for structural determinations.

The third section describes the isolation of aroma concentrates from heated rapeseed oil and the separation of the concentrates into their components by gas chromatography followed by organoleptic evaluation and chemical identification.

- A -

GLASS CAPILLARY COLUMNS

WITH POLAR PHASES AND SILANOX

INTRODUCTION

This section contains a review of the methodology and technological developments leading to the production of support coated open tubular columns, a description of the methods used in this study for the extrusion and the coating of glass capillaries, a description of the conversion of a gas chromatograph to an all-glass system, and an evaluation of the columns produced and the performance of the all-glass system.

German and Horning (1973) have reported a two-step dynamic coating procedure for the production of support coated open tubular columns with Silanox and apolar stationary phases. By a novel technique recently published by McKeag and Hougen (1977a) and reported here in greater detail, the method of German and Horning (1973) has been extended to include the use of polar stationary phases.

LITERATURE REVIEW

This review summarizes the advances in the theory and technology of gas chromatography leading to the production of support coated open tubular columns.

Column Theory

Since the invention of gas-liquid chromatography by James and Martin (1952), the popularity of this method of analysis has been enhanced by improvements in instrumentation and technology produced through diligent research. Specific and sensitive detectors, solid state electronics, thermostable liquid phases of many different degrees of polarity, temperature programming, and derivatization techniques are some of the major innovations utilized in modern gas-liquid chromatography.

The basis of separation in the gas-liquid chromatograph is the distribution of a vapor sample between a moving carrier gas and a stationary liquid phase. The separation takes place in the "heart" of the gas chromatograph — the column. A brief review of gas chromatographic column evolution follows.

Early columns were simply metal tubes packed with inert particles supporting liquid films. For packed columns, efficiency, or separating power, was first described in mathematical terms by van Deemter (1956). Equation 1 expresses the theoretical plate height as a function of linear gas velocity:

$$h = A + B/\bar{u} + (C_g + C_l)\bar{u} \qquad [\ 1\ ]$$

where h is the height equivalent to a theoretical plate,

$\bar{u}$ is the mean linear velocity of the carrier gas, and

A, B, C_g and C_l are constants describing contributions to plate height made by eddy diffusion, longitudinal gaseous diffusion, and resistance to mass transfer in the gaseous and liquid phases, respectively.

At the Second International Gas Chromatographic Symposium, M. J. E. Golay (1958) proposed a chromatographic column with a liquid phase coated on the inner wall of the tubing. He realized that a major limitation to higher efficiency in packed columns was the slowness of diffusion of sample components within the pores of the chromatographic packing. On a theoretical basis, he postulated that higher efficiencies should be attainable with a column which provided an unrestricted open path for the gases to flow.

Golay's equation for open tubular column efficiency may be written:

$$h = B/\bar{u} + (C_g + C_l)\bar{u} \qquad [\ 2\ ]$$

The higher efficiency of open tubular columns than for packed columns may be attributed to two factors, namely, the elimination of eddy diffusion and an appreciably reduced resistance to mass transfer in the gas phase.

The next major advance in column technology was the development of support coated open tubular (SCOT) columns by Golay (1960). An expanded form of the C_l term in equation [2] was then introduced:

$$C_l = \frac{2k}{3(1+k)^2} \quad \frac{d_f^2}{D_l} \qquad [3]$$

where k is the partition ratio,

d_f is the film thickness, and

D_l is the diffusion coefficient of the solute in the liquid phase.

It is evident that by reducing the film thickness, or increasing the partition ratio, the contribution of the C_l term to plate height will decrease. The partition ratio will only be increased if the column diameter is decreased or if the film thickness is increased. The latter possibility is in direct conflict with the previous objective, i.e., reduction of the film thickness, while the first possibility has obvious practical limitations.

Golay's proposed solution to this problem was simple yet effective, namely, to coat a bed of material that was affixed to the wall of the tubing. Like open tubular columns, an unrestricted path for gas flow would be preserved, but larger amounts of liquid phase could be coated and maintained as a thinner film on the support material.

Column Technology

The development of open tubular columns may be considered to be one of the most important contributions made to the field of chromatography. A further advancement with open tubular columns was the invention of a glass capillary drawing machine by Desty *et al.* (1960). Glass offers properties superior to other available column materials. The inertness of glass reduces adsorption losses, chromatographic tailing, and the production of artifacts through rearrangements

or degradations.

The manufacture of efficient glass open tubular columns suitable for the high resolution required for separation of complex mixtures has been the objective of many laboratories. The development of glass open tubular columns has been difficult because of the fundamental problem that liquid films, especially of polar stationary phases, tend to be nonuniform and unstable when coated on smooth glass surfaces.

A successful approach to coating glass surfaces is to develop a microirregular layer on the glass as a means of supporting a uniform stable liquid film. General methods of surface modification include etching, coating with a porous material, and embedding some material in the glass. A stationary liquid phase is then coated or chemically bonded to the modified surface. Comprehensive reviews on the techniques used for surface modification of glass and coating of capillary columns include those of Novotný and Zlatkis (1971) and Ettre and Purcell (1974). Current developments are compiled in a special issue of the journal Chromatographia (1975).

The deposition of an adhering layer of particles to form a microirregular surface, thereby increasing the amount of liquid phase which may be coated, is the basis of support coated open tubular (SCOT) columns. A new concept, reported sequentially by German and Horning (1973) and by Blumer (1973), involves the use of coating solutions containing Silanox 101. This material is hydrophobic silica with a primary particle size of about 7 mμ.

The purpose of adding Silanox to a solution of liquid phase used for coating smooth glass surfaces is to preserve film continuity. The

film continuity is maintained by interconnected pools of liquid phase supported in a matrix of Silanox particles. If the distribution of Silanox is sufficienty regular, a stable film is maintained and the formation of microdroplets of liquid phase is highly improbable.

The dynamic method of coating with Silanox suspensions, used by German and Horning (1973), produces highly efficient thermostable glass SCOT columns with apolar liquid phases. A limitation of this method, reported by Van Hout *et al.* (1974) and Lin *et al.* (1975), is that the dynamic method produces uneven coatings and correspondingly low column efficiencies when applied to polar liquid phases. To overcome this difficulty, these authors deposited beds of Silanox containing small amounts of the polar liquid phase on glass surfaces by dynamic coating. Additional polar liquid phase was coated on the bed by static methods. Blakesley and Torline (1975) have reported successful dynamic coating of glass capillaries with polar liquid phases and Silanox, using the surfactant Igepol CO-880. The use of surfactants, however, has been reported by Jennings *et al.* (1974) to change the retention and possibly the order of elution of compounds.

Another method of obtaining a stable stationary phase film on the glass surface is direct chemical bonding of the phase to the glass. This approach has been used to obtain highly stable and efficient packings from silica particles for conventional columns. Bonding reactions involve reagent combinations with surface silanol groups. Halasz and Sebestian (1974) have reviewed esterification reactions producing such "brush type" stationary phases. A modification of this technique has been patented by Bossart (1970). The chemically etched

wall of a glass capillary tube was reacted to bond a fixed stationary phase through an ester linkage. No chromatographic applications were reported. The application of this technique to produce bonded phase glass capillary columns for gas chromatography and their application to chromatographic separations has been reported by Einig and MacDonald (1976).

Procedures for bonding alkyl and aryl substituents directly to silicon on silica surfaces have been reported by Locke *et al.* (1972). The direct Si-C bonds obtained by this method exhibit much higher thermal and hydrolytic stability than silicon ester bonds.

Methods of obtaining silica layers on glass surfaces through heat induced microphase development have been reported by Haller (1965) and Porai-Koshits and Aver'yanov (1973). No reports in the literature have been found concerning the possible use of microphase development as a means of surface modification prior to chemically bonding a substrate for open tubular gas chromatography.

MATERIALS AND METHODS

This section describes procedures for the extrusion and coating of glass capillary tubing to produce support coated open tubular columns and the conversion of a gas chromatograph to an all-glass capillary system.

Glass Tubing Preparation

Duran 50 glass tubing, 5.0 ft x 7.0 mm o.d. with 1.0 mm wall, was utilized. Extensions were joined to the ends of the tubing to allow extrusion of the whole length. A 5.0 mm o.d. glass tube, 10 cm long, was inserted 1 cm into one end of the tubing and fused with a burner flame. A 7.0 mm o.d. glass tube, 20 cm long, was fused to the other end of the tubing by butting the ends of the tubes together guided by a glass insert 1.5 cm long. Before solidification occurred, the fused tube was rolled on a flat surface to assure straightness of alignment.

Cleaning Glass Tubing

To allow smooth even extrusion of glass tubing, all glass must be scrupulously cleaned prior to drawing. Glass tubes were cleaned in the following manner. Tubes were rinsed with hot water, washed with detergent, and rinsed again with hot water. During the remainder of the glass cleaning procedure, polypropylene gloves were worn to prevent fingermarking the glass surface and for protection of the hands. The tubes were immersed for 2 hr in 1.0N NaOH saturated with $KMnO_4$ and

agitated occasionally.

CAUTION — Caustic $KMnO_4$ is a powerful oxidizing agent which burns skin on contact.

After rinsing with distilled water, the tubes were immersed in concentrated HCl for 1 hr and agitated occasionally. After successive rinses with distilled water and absolute methanol, the tubes were dried by simultaneous water aspirator suction and hot air from a heat gun.

Glass tube reservoirs, 1.65 m x 35 mm i.d., were used for the corrosive caustic $KMnO_4$ and concentrated HCl solutions. Four tubes were cleaned simultaneously. Rinse operations were performed with polystyrene squeeze bottles. After drying, the clean tubes were stored in a sealed tubular cardboard container.

Extruding Glass Capillary Tubes

A Hupe and Busch 1045A capillary drawing machine was used with a 12-cm diameter coiling tube. The glass tubing to be extruded was guided to the feed rollers of the drawing machine through a 3 ft long x 10 mm i.d. glass tube supported by two laboratory stands. The furnace and coiling tube were set at 78 and 75, respectively, and allowed to equilibrate for 15 min. During this time, the axles of the upper rollers of the feed and draw mechanisms were lubricated with graphite powder and tightened to assure proper alignment. Spring tensions in the feed and draw mechanisms were adjusted to assure continuous smooth extrusion. The coiling tube was lubricated with graphite by blowing the powder into the tube with a disposable pipette.

If long capillaries were to be drawn (> 60 m), an extension was added to the receiver rod.

When the furnace and coiling tube temperatures had stabilized, the tubing was advanced manually until about 1 cm of the tubing to be drawn was in the hottest zone of the furnace. After about 1 min, when the tubing became ductile, it was gripped with needle-nosed pliers, extruded manually and guided through the draw rollers. The draw rollers were then activated using a draw ratio of 14. The extruded capillary was immediately guided to the deflector notch and about 2 m of capillary tubing was drawn. Once the diameter of the capillary tubing appeared to be regular, the tubing was broken off at the draw rollers and guided into the coiling tube, and the length counter was set to zero.

The extruded capillary tubing was then continually lubricated with graphite by placing a graphite reservoir between the draw rollers and the coiling tube. The reservoir was fabricated from a hollow cylindrical container, 8.5 cm high and 4.0 cm diameter. Slots, 4 mm wide, were cut 2.0 cm deep across the cylinder face. When the cylinder was filled with graphite and placed in position, the extruded capillary tubing passed through the slots carrying a constant stream of graphite lubricant into the coiling tube. The reservoir was filled with additional graphite as required. The coiling tube was tapped occasionally with a glass rod to loosen the graphite to promote smooth continuous coiling.

When the extrusion was completed, a 5-cm length of tubing was removed from the coil and retained for internal diameter measurement. After sealing the ends with a torch flame, the coil of tubing was carefully immersed in running water to remove adhering graphite which

readily floated to the surface. The tubing was then rinsed with successive portions of ethanol and acetone. The clean tubing was air dried on a rod supported on a laboratory stand. The tubing was kept sealed until being coated.

The internal diameter of the glass capillary tubing was measured with a Nikon Profile Projector, model 6C, used in the shadow mode with 10X magnification. A 2-3 mm long section of tubing was measured, mounted standing on a microscope slide fixed with transparent tape having adhesive on both sides.

Bending Coiled Glass Capillaries

The tubing was supported on a metal rod affixed to a laboratory stand. A hand torch was used with luminous flame about 2.5 cm long. The tubing was aligned in such a manner that when heated to its melting range, it fell under its own weight into the desired configuration.

Preparation of Support Coated Open Tubular (SCOT) Glass Columns

The glass capillary tubing was coiled about a cylinder, 1.25 m x 11.9 cm o.d., supported horizontally on laboratory stands. When the coils had been evenly spaced over the cylinder, the glass tubing was affixed to the cylinder with two 2.5-cm wide lengths of paper tape.

The cylinder supporting the glass capillary tubing was mounted vertically on a laboratory stand. The upper end of the glass capillary tubing was connected to a coating solution reservoir, and the lower end to a flow restrictor consisting of a 10-15-m piece of coiled glass capillary tubing having a diameter identical to the tubing to be coated. A glass capillary tube ready to be coated is shown in Figure 1.

The coating solution reservoir was fabricated from a 28-cm long

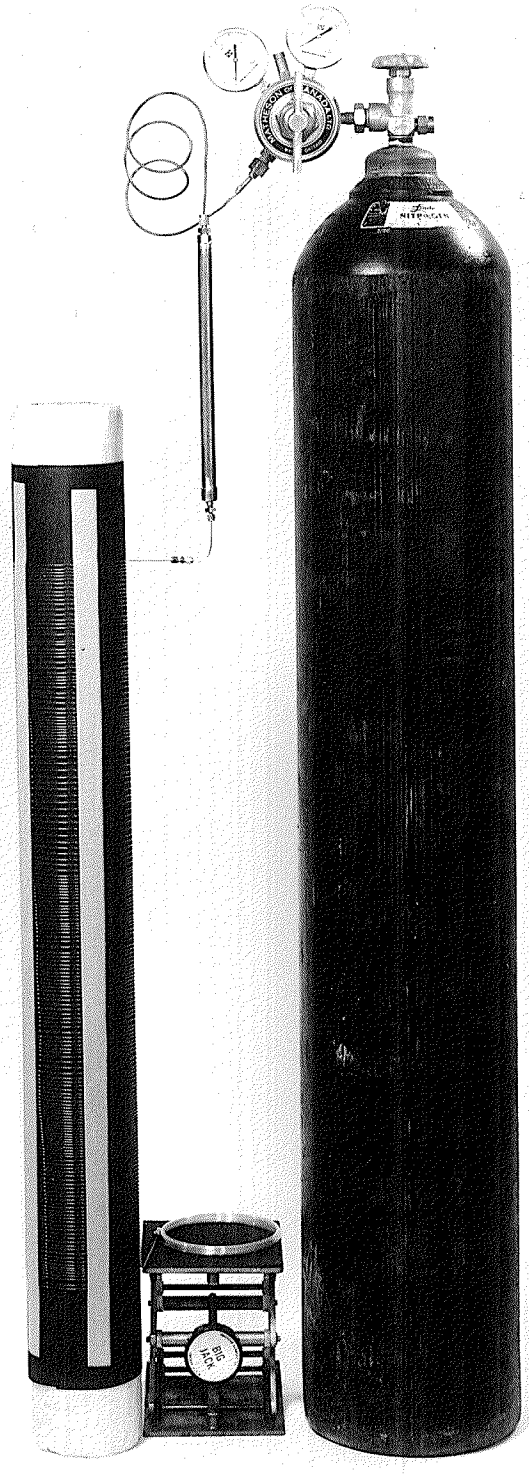


Figure 1. Apparatus for coating glass SCOT columns.

x 1.50-cm o.d. copper tube. A 0.25-in. stainless steel Swagelok nut was silver-soldered to one end of the tube to allow connection to a gas cylinder via a union. A copper cap was silver soldered to the other end of the tube. The centre of the cap was bored through with a 0.25 in. bit. The wider end of a 1/8-1/16-in. stainless steel Swagelok reducing union was inserted into the hole in the cap and silver soldered.

All connections between metal parts and glass capillary tubing were made with standard 1/16-in. Swagelok fittings sealed with a teflon washer. A strip of teflon tape about 2.5 cm. x 3.0 mm was wound about the tubing between the back ferrule and nut. When the nut was tightened, compression caused the teflon to flatten into a washer which sealed the connection.

The coating procedure reported here for the production of a capillary column was used with the liquid phases OV-225, OV-210, OV-101, Silar-5CP and Carbowax 20M TPA. The coating technique used was a modification of a two step dynamic process first described by German and Horning (1973). The solvent used for both solutions required for the coating procedure was chloroform:acetone (10:1). Chloroform and acetone were distilled prior to use.

CAUTION: As reported by King (1970), chloroform-acetone mixtures may be highly explosive. In the presence of basic substances, a catalytic, highly exothermic condensation reaction takes place.

In the first step, solvent, 5% of the column,volume was pipetted into the reservoir. The reservoir was connected to a nitrogen cylinder

and the solvent propelled into the glass capillary tubing under 5 psi gas pressure. When the plug of liquid had been expelled into the tubing, the pressure was reduced to zero by disconnecting the gas line from the reservoir.

A suspension, formed by the addition of 0.25 g Silanox (Grade 101) to a solution of 0.10 g of a stationary phase dissolved in 10.0 ml of solvent, was sonicated 1 min. An aliquot, 20% of column volume, was funneled into the reservoir with a thistle tube, and 80 psi gas pressure was applied to propel the solution at about 18 cm/sec through the tubing. When the coating was dry, as indicated by an irregular opaque white appearance, the gas pressure was reduced to about 5 psi and maintained overnight.

In the second step, the layer deposited on the glass tubing was coated with a solution of 0.25 g of the stationary phase dissolved in 10 ml of solvent. An aliquot, 20% of column volume, was pipetted into the reservoir and 45 psi gas pressure was applied to propel the solution at 12-15 cm/sec through the tubing. When the main plug of solution had been expelled, the gas pressure was reduced to 30 psi to expel some remaining smaller plugs of liquid. A further reduction in pressure to about 10 psi promoted smooth flow of highly viscous secondary plugs of coating material formed by solution draining from the column walls. When the coating was dry, as indicated by a uniform opaque appearance, the gas pressure was reduced to 5 psi and maintained overnight.

Connection of the SCOT Column to the Gas Chromatograph

The glass SCOT column was supported on a stand constructed from two aluminum plates, 5.5 x 5.5 x 1/8-in. thick, and three threaded stainless steel rods, 5/32 in. o.d., 8 in. long. Holes were drilled through the plates equally spaced on the circumference on an 11.9-cm diameter circle and the plates fixed to the ends of the rods between pairs of stainless steel nuts.

A glass inlet and stainless steel coupling were fabricated to permit splitless injection to a glass SCOT column. The device is shown in Figure 2. A capillary bore (1.0 mm i.d.) glass tube, 6.3 mm o.d., 15.4 cm long, was ground flat at the ends with a carborundum grindstone. To facilitate injection, the internal diameter of one end of the tube was enlarged with a carborundum bit to about 2.0 mm. This tube was inserted into a standard 0.25-in. injector port of the chromatograph and affixed with 0.25-in. teflon ferrules.

The glass SCOT column was connected to the glass insert via a modified 1/4 in.-1/8 in. stainless steel Swagelok reducing union. The 1/8-in. connection was cut off with a hacksaw at the centre nut and the surface filed flat. The 1/4-in. side of the union was bored out to accept 14 mm of the glass insert. A special bit was machined to remove any taper at the bottom of the hole bored into the union.

The capillary tubing was inserted through the union and a 6.2 mm diameter disc of Pierce Stabiline septum. The insertion of the glass capillary through the septum material was accomplished by piercing the septum with a syringe needle, inserting the glass capillary into the needle and through the septum, and removing the needle. The 2-3 mm

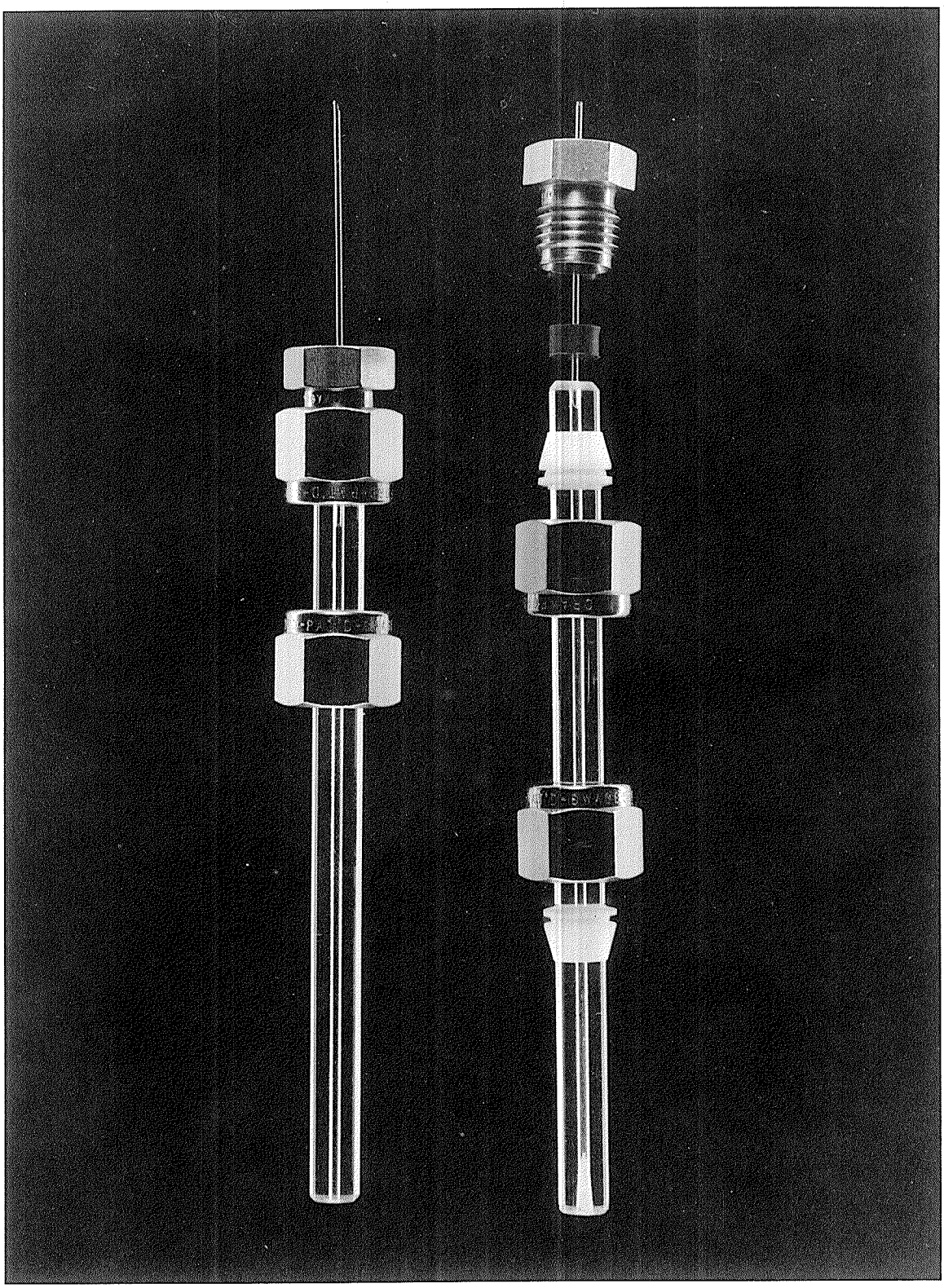


Figure 2. Glass inlet and coupling for splitless injection.

of capillary tubing protruding through the septum disc was inserted into the glass injector tube. The modified union was then coupled to the injector tube with a 0.25-in. stainless steel nut and teflon ferrules. Care was taken to hold the modified union tightly against the injector tube while the nut was tightened to ensure adequate compression of the septum disc against the insert and to prevent breakage of the capillary tubing from torsion.

An injection guide was fabricated to facilitate direct injection into the glass injector insert. The centre of a 3/8-in. o.d. brass rod was bored to a depth of about 0.5 in. with a 0.040-in. bit using a metal lathe. A washer, about 3/64 in. thick, was cut from the rod. A 25 mm length of number 18 stainless steel syringe needle was inserted into the brass washer and silver soldered. This guide was inserted in front of a septum and affixed to the gas chromatograph with the septum cap.

The column was connected to the chromatograph detector with a 1/16-in. stainless steel Swagelok union. A gas tight seal between the metal union and glass column was obtained with a teflon washer and ferrules combination previously described.

Column Conditioning

After installation of the column in the gas chromatograph oven, the helium carrier gas flow rate was set to 4.0 ml/min (about 35 cm/sec). The flow rate was determined with a conventional soap film flowmeter by inserting the outlet end of the glass capillary column through a pierced rubber serum cap affixed to the flow meter tubing.

The column was purged with helium for 20 min at 30^{o}C and then the

column temperature was increased at 2°C/min to 250°C and maintained at 250°C for 12 hr. The column temperature was then decreased to 200°C and the column was silanized with four injections of 2.5 µl of Silyl-8 injected at 5-min intervals. After maintaining the column at 200°C for an additional hour, the column was ready for use.

RESULTS AND DISCUSSION

This chapter discusses the extrusion and coating of glass capillaries to produce support coated open tubular columns. This is followed by an evaluation of the modifications made to a gas chromatograph to produce an all-glass capillary system and an evaluation of the various capillary columns produced.

Extrusion of Glass Capillary Tubing

The Hupe and Busch 1045A capillary drawing machine should be installed in a draft-free area because the coiling tube is very sensitive to temperature changes. Prior to extruding tubing, the drive motor gear box cover should be temporarily removed and the draw mechanism activated for short periods to test that the gears are properly engaged. This precaution will insure that the gears will not be damaged or the drive motor burned out.

The glass tubes had to be supported and guided through a fixed glass tube to the feed rollers of the capillary drawing machine. The tension in the feed roller spring was insufficient to guide the tubing to the centre of the furnace. The slightest misalignment of the tubing entering the furnace will result in uneven heating and the extrusion of irregular capillaries.

The capillary drawing machine was tested by extruding glass tubing of various outer diameters and wall thicknesses. It was determined that 7.0 mm o.d. tubing with 1.0 mm thick wall was the optimum size for

extruding capillaries having internal diameters of 0.35 to 0.80 mm. Extruding tubing with walls thicker than 1.0 mm proved to be exceedingly difficult; the high furnace temperatures necessary to melt the glass produced irregular capillaries.

When the furnace and draw ratio had been set for smooth even extrusion of capillary tubing, the draw ratio could only be changed ±1 unit without having to also alter the furnace temperature. The furnace temperature and draw ratio should be simultaneously adjusted for optimum performance.

The uniformity of extruded tubing was determined by measuring the internal diameters of capillaries drawn from Duran 50 tubing, 7.0 mm o.d., 1.0 mm wall. The oven and coiling tube rheostats were set at 80 and the draw ratio set to 14. After discarding the first 2.0 m of coiled capillary tubing, 1-cm samples were removed from the ends of 10 succeeding 3.0-m coils. Measurements with the Nikon profile projector showed the mean internal diameter of the coiled capillary to be 0.624 mm with a standard deviation of 0.001 mm.

Contrary to results obtained by Desty *et al.* (1960), drawing sections of tubing joined by glassblowing resulted in irregular or broken capillaries. Because long capillaries were desired, Duran 50 tubing was selected; this tubing was available in longer standard lengths than other glasses. Duran 50 is a chemically resistant sodium borosilicate glass.

Smooth continuous coiling of long glass capillaries was greatly improved by the addition of a graphite reservoir placed between the draw rollers and the coiling tube of the drawing machine. A similar device has been reported by Reiner and Weaver (1974).

Coating Glass Support Coated Open Tubular (SCOT) Columns

This section describes the attempted chemical bonding of a stationary phase to the walls of a glass capillary tube and the successful coating of SCOT glass columns with liquid phases and Silanox.

Chemical Bonding of a Phase to a Modified Glass Surface

In a preliminary study of coating glass capillaries, an attempt was made to chemically modify the glass wall to allow direct bonding of a phase. This approach was adopted because of claims made by Halasz and Sebestian (1969) and Little *et al.* (1970) regarding the thermostability and efficiency of liquid phases bonded via Si-O-C linkages to silica beads used for packing material in conventional columns for gas chromatography.

If alkali borosilicate glasses are tempered over extended periods of time at temperatures of 560-600^{o}C, a separation of microscopically small dimension occurs. The initially homogeneous glass (one phase) separates into two new phases, namely, one rich in silica and another rich in alkali borate. This process, described by Haller (1964), has been attributed to the spontaneous growth of nucleation centers upon cooling the system below a characteristic immiscibility temperature.

Glass capillaries were heated in a Gallenkamp Model FR-572 muffle furnace at temperatures of 565, 585, and 605^{o}C, each for periods of 4, 8, 16, and 48 hr. Viewing of glass samples with a Cambridge Steroscan Mark II scanning electron microscope revealed that no microphase separation occurred. In addition, the thin walled capillary tubing coil deformed at all treatment temperatures in spite of attempts to support the coils on a 12.0 cm diameter ceramic cylinder. As the

deformation point of Duran 50 glass is 610°C, the failure to obtain microphase separation might be attributed to uneven heating of the glass due to temperature gradients within the furnace.

If phase separation had occurred on the glass surface, the alkali borate phase could have been etched from the surface with HCl. A second heat treatment and etching should have produced a further enrichment of silica on the glass surface.

It was intended that surface silica be reacted to obtain surface silanol groups. Surface silanol groups could then be reacted via the method of Locke *et al.* (1972) to yield chemically bonded alkyl or aryl substituents:

$$\equiv \text{Si - OH} \xrightarrow{\text{SiCl}_4} \equiv \text{Si - Cl} \xrightarrow{\text{RMgBr}} \equiv \text{Si - R}$$

Direct Si-C bonds exhibit superior thermal and solvolytic stability compared to Si-O-C bonds.

A phase chemically bonded to glass, having mechanical, thermal, and solvolytic stability, would have considerable impact upon the gas chromatographic technique. The elimination of stationary phase bleed and drifting baselines would lead to better electronic integration and allow the use of detectors at higher sensitivities. Structural elucidation of gas chromatographic effluents by gas chromatography-mass spectrometry would be simplified by the absence of background from liquid phase contamination.

Although microphase separation failed to produce a suitably modified glass surface for further chemical reaction, the concept of a chemically bonded liquid phase has merit. The modification of glass

surfaces to produce surface silanol groups by chemical or physical methods followed by chemical reaction to form ≡Si-R bonds is worthy of further investigation.

Coating Glass SCOT Columns with Liquid Phases and Silanox

The concept of producing and maintaining a continuous liquid film supported by a matrix of minute silanaceous particles is remarkable for its simplicity. This technique, first proposed by German and Horning (1973) for the production of apolar phase columns, has the advantage that a simple dynamic coating method is used. The preparation of efficient polar-phase glass capillary columns by a modification in the solvent system used in the German-Horning dynamic two-step coating procedure has been reported by McKeag and Hougen (1977a).

Initial attempts at coating 60-85-m lengths of 0.62 mm i.d. glass capillaries with a suspension of Silanox in a CCl_4 solution of OV-101 liquid phase or a suspension of Silanox in a $CHCl_3$ solution of OV-225 liquid phase both resulted in grossly irregular coatings or plugged capillaries. These coating solvents were those reported by German and Horning (1973). Coating solutions were highly viscous and had a tendency to form a thixotropic gel. Regular flow rates could not be maintained during the coating process. Gas pressures up to 240 psi were necessary to induce flow in columns containing gelled coating suspensions.

It was apparent from the reports of German and Horning (1973) and Blumer (1973) that two conditions were required to obtain uniform coatings with Silanox suspensions. A high density solvent should be used to stabilize the suspension, and the solvent should be of high polarity

to inhibit gel formation. The chloroform-acetone solvent system was chosen because of the high density of chloroform and the high polarity and low relative viscosity of acetone.

The concentrations of liquid phase and Silanox used in the first coating step were found to be critical. A solution containing 0.10 g stationary phase and 0.25 g Silanox in 10.0 ml solvent successfully coated the entire glass surface when propelled through the capillary tubing. Applied gas pressures of 60-80 psi maintained uniform plug velocities with the Silanox suspensions. Larger concentrations of Silanox or liquid phase promoted gel formation, while smaller concentrations resulted in incomplete coverage of the surface.

Irregularities in the Silanox bed deposited during the first coating step had little effect upon final column performance provided that the entire glass surface was covered with the Silanox-liquid phase combination. Irregularities in the thickness of the Silanox bed could be seen after the first coating step; these irregularities, however, were eliminated during the second coating step. The localized patches of excess Silanox were apparently washed away, leaving a uniform thin layer of Silanox, which was estimated by Scanning Electron Microscopy to be 2.5 μm thick. This value of layer thickness was similar to that reported by Schieke *et al.* (1975) who reported that a Silanox layer deposited on the wall of a glass SCOT column was not uniformly thick, but had an average thickness of about 6 μm.

During the second coating step gel formation was never observed. Constant plug velocities of 12-15 cm/sec were easily maintained with applied gas pressures of about 45 psi. Once the main plug of coating solution

had been expelled, the gas pressure was reduced in stages to promote smooth flow of the secondary plugs formed by solution draining from the column walls. Failure to reduce the gas pressure during the last stage of the second coating step resulted in the break up of the secondary plugs with spattering on the column walls.

Bed surfaces obtained with and without gas flow reduction in the final stage of coating a glass capillary with Silanox and Silar-5CP liquid phase are shown in Figure 3. While the surface obtained by coating with gas flow reduction is regular in appearance, the surface obtained without flow reduction appears to be a double layer containing numerous fissures. Spattering of the coating solution under the latter condition produced a visibly heavier coating which developed fine cracks after conditioning of the column. Capillaries with thickly coated beds containing fissures gave poor chromatographic separations.

Silylation of columns during the conditioning procedure with Silyl-8 reduced chromatographic tailing of polar compounds. This may be attributed to the silylation of silanol groups on the Silanox particles. This view is substantiated by the tendency of the coating solution to form gels since the mechanism of gel formation with Silanox is attributed to hydrogen bonding between surface silanol groups on adjacent particles.

Contrary to results obtained by German and Horning (1973), silylation of the glass surface prior to coating proved to be detrimental to the coating procedure; with this procedure the Silanox suspension would not adhere to the glass surface.

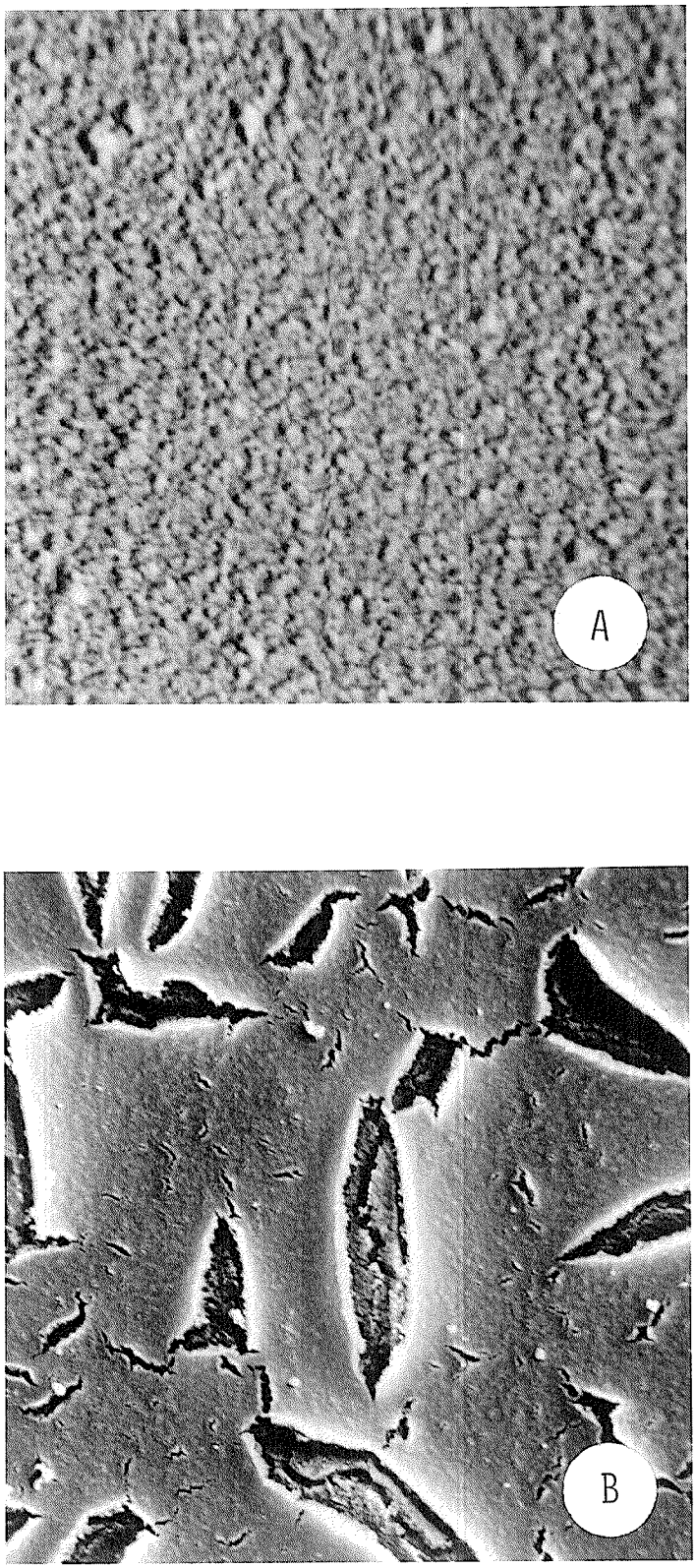


Figure 3. Micrograph of a Silanox bed coated (A) with pressure reduction during coating and (B) without pressure reduction. Magnification about 500X.

Instrument Modifications for an All-Glass System

The primary objective in the design of the system was the achievement of an inert system in which even unstable compounds could be delivered to the FID detector or splitter, after separation, for simultaneous chromatographic and organoleptic evaluation.

The glass injector design used was in principle similar to that proposed by Verzele *et al.* (1972). This simple design may be easily adapted to any gas chromatograph having a heated injector port.

The high efficiency of the injector system may be attributed to the inertness of the glass insert and the minimal dead-volume of the column coupling. When used in conjunction with larger bore capillary columns, i.e., 0.5 mm or larger, splitless injection results in only small losses in efficiency when compared to injection with split. The advantages of splitless injection are twofold; superior quantitative data are obtained as the non-linearity of splitters is avoided, and the relatively large samples chromatographed allow the detection of trace components.

In a chromatographic system, the injector port may be a major site of sample degradation. The sample degradation occurs during the vaporization process while the liquid is in contact with catalytically active metal parts. Once vaporized, the sample is relatively stable. For these reasons, the columns used in this study were coupled to a glass injector insert and to a metal inlet tubing of the gas chromatograph detector. When extremely labile compounds are being chromatographed and an all-glass system is preferred, the injector insert and coupling unit may be adapted to connect the glass capillary to the

chromatograph detector.

Evaluation of Column Performance

Columns were successfully prepared with Silanox and the polar liquid phases Silar-5CP, OV-210, OV-225, and Carbowax 20M TPA, and with the apolar liquid phase OV-101. All columns exhibited excellent efficiencies and thermostabilities. The columns displayed high durability, tolerating splitless injection of flavor volatiles and fatty acid methyl esters for 6 months before appreciable losses in efficiency occurred.

Typical characteristics of dynamically coated glass SCOT columns are shown in Table 1. Column efficiencies were tested by splitless injection of 0.10 µl of *circa* 2% test substance in a suitable volatile solvent. Helium carrier flow rates and the gas-holdup times were determined by injection of natural gas, i.e., a non-sorbed solute. The Silar-5CP and OV-101 columns were tested in a Varian Aerograph Model 1800 gas chromatograph and the Carbowax 20M TPA column in a Perkin Elmer 3920B chromatograph. Both instruments were equipped with FID detectors.

The two-step dynamic coating process was highly efficient, producing columns with greater than 60% of the theoretical maximum number of plates attainable. On a routine basis, capillary columns having lengths of 50-135 m could be coated with approximate effective efficiencies of 800-1000 plates per meter. However, the calculated number of theoretical plates may not be an accurate indication of the efficiency of a column, as many factors such as sample size, retention time of a compound, and dead volume of the chromatographic system significantly influence the efficiency.

TABLE 1. Characteristics of dynamically coated SCOT columns with Silanox.

		Liquid Phase		
		Silar-5CP	Carbowax 20M TPA	OV-101
Length	(m)	135.0	52.5	131.7
Column i.d.	(mm)	0.62	0.62	0.50
Temperature	(^{o}C)	175	175	180
Flow rate	(cm/sec)	22.1	32.9	25.1
Inlet pressure	(kg/cm^2)	2.50	2.50	2.50
Capacity ratio		9.10	3.50	2.77
HETP	(mm)	0.894	0.773	0.525
$HETP_{eff}$	(mm)	1.11	1.28	0.973
Coating efficienty	(%)	61.6	64.4	73.7
Test substance		methyl-linolenate	*iso*-propyl-palmitate	*n*-hexa-decane

Chromatograms showing the separation of a complex mixture of hydrocarbons with an OV-101 column and the separation of an aroma isolate with a Silar-5CP column both illustrate the characteristics and performance of SCOT columns coated with liquid phases and Silanox. Column dimensions and flow rates are listed in Table 1.

The chromatogram showing the analysis of regular grade gasoline is shown in Figure 4. The chromatograph range was set at 10^{-11} afs and the attenuation adjusted as indicated on the chromatogram. After splitless injection of 0.10 µl of the sample, the column temperature was

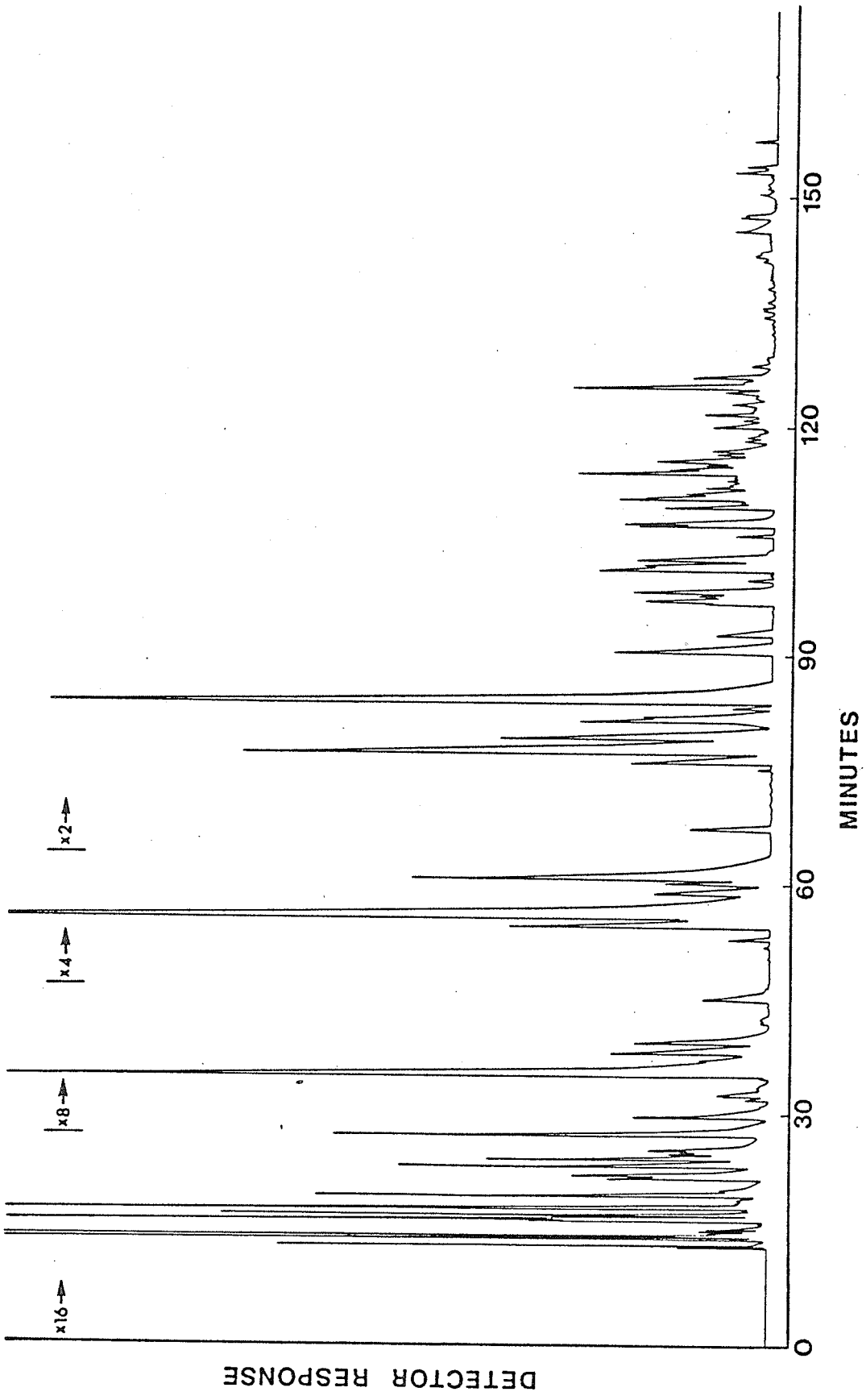


Figure 4. Gas chromatogram of regular grade gasoline.

maintained at 25°C for 20 min and then sequentially increased at 1°C per min for 55 min, 2°C per min for 38.5 min, and 4°C per min for 16.5 min, and maintained at 223°C until the end of the analysis.

The OV-101 column exhibited excellent efficiency; 163 components were resolved. The peak symmetry, for all but a few components of very high concentration, reveals the high sample capacity of the column. The thermostability of the column is evident as no baseline shift caused by column bleed is seen in spite of the high sensitivity of the electrometer setting.

The chromatogram of an odor isolate from heated rapeseed oil is shown in Figure 5. After splitless injection of 0.1 µl of the sample, the column temperature was maintained at 25°C for 10 min and then sequentially increased at 1°C per min for 99 min, and 2°C per min for 11 min, and maintained at 146°C until the end of the analysis. The chromatograph attenuation and range were set at 8×10^{-10} afs.

The Silar-5CP column exhibited excellent efficiency and thermostability. The odor concentrate was resolved into 138 components. During GC-MS analysis only minimal column bleed was detected. Splitless injection of 0.30 µl of odor isolate combined with post-column splitting for organoleptic evaluation of the components produced only a slight decrease in peak resolution. Although many of the compounds in the odor isolate were of very polar character, i.e., alkenals, alcohols, etc., chromatographic tailing was minimal. This suggests that the Silanox matrix maintained on the glass capillary surface acted as a highly inert support for the stationary phase liquid film.

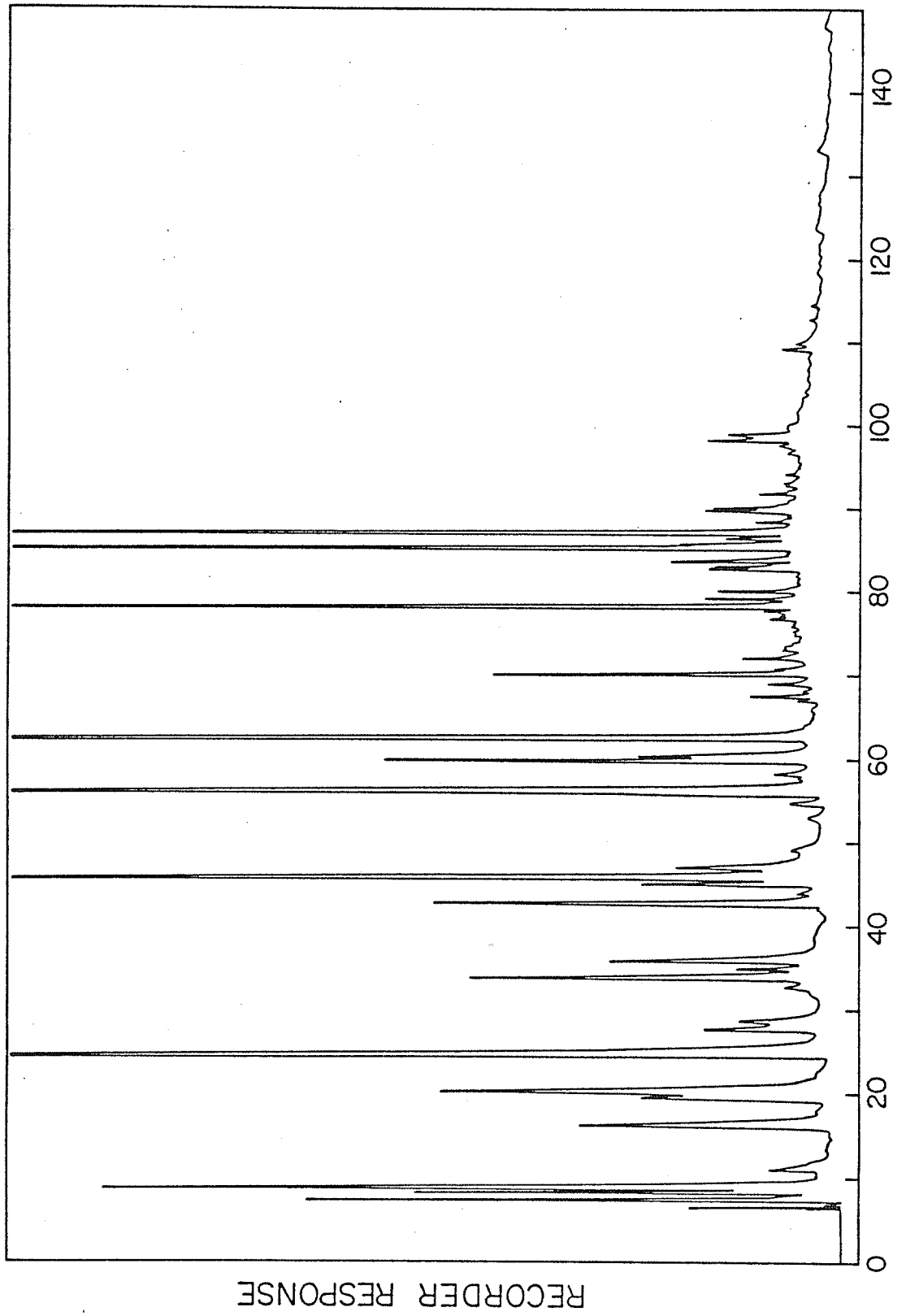


Figure 5. Gas chromatogram of odor isolate from heated rapeseed oil.

During a study of the odor isolate from heated rapeseed oil three glass Silar-5CP SCOT columns were produced. These columns, having lengths of 106.5, 125.0, and 135.0 m and 0.62 mm i.d., gave very similar separations of the odor isolate. Although no direct comparison of retention data and column efficiencies was attempted, it appears that the coating method allows production of columns having reproducible properties.

SCOT columns prepared with OV-225, OV-210, and Carbowax 20M TPA liquid phases exhibited characteristics similar to those described for the OV-101 and Silar-5CP columns. High efficiency, thermostability, and sample capacity and minimal chromatographic tailing were common characteristics of the glass SCOT columns produced with Silanox.

SUMMARY AND CONCLUSIONS

A Hupe-Busch 1045A glass capillary drawing machine was used to extrude and coil highly uniform bore glass capillaries from Duran 50 glass tubing. Important factors in the extrusion and coiling of glass capillaries were found to include scrupulous cleaning of the tubing to be drawn, support and precise alignment of the tubing with the feed roller system, and simultaneous adjustment of the furnace and coiling tube temperatures for different draw ratios. Capillary coiling was aided by the addition of a graphite reservoir to provide continuous lubrication.

The solvent mixture reported in this work has extended the German-Horning (1973) procedure for dynamic two-step coating of glass SCOT columns with Silanox to include the use of polar liquid phases. A uniform thin layer of Silanox about 2.5 μm thick, supporting a stationary phase liquid film, was dynamically deposited on an untreated glass capillary wall from chloroform-acetone coating solution.

In the first coating step, a bed of Silanox was deposited on the glass capillary wall. The chloroform-acetone (10:1) solvent combination had adequate density to stabilize the Silanox coating suspension and sufficient polarity to inhibit thixotropic gel formation. At optimum concentrations of 0.25 g Silanox and 0.10 g liquid phase in 10.0 ml of solvent, complete though slightly irregular coverage of the glass surface was obtained.

Additional liquid phase was dynamically deposited on the Silanox bed during the second coating step. The coating solution, containing 0.25 g liquid phase in 10.0 ml of solvent, appeared to wash away the Silanox bed irregularities produced in the first coating step. When the main plug of coating solution had been expelled, a gradual reduction of the applied gas pressure promoted smooth flow of the viscous secondary plugs of coating solution. This procedure produced highly uniform thin layers of Silanox which supported the liquid phase.

Columns prepared with Silanox and the liquid phases OV-225, OV-210, OV-101, Silar-5CP, and Carbowax 20M TPA have shown excellent efficiency and thermostability. Effective plate heights of about 1.0 mm were obtained with column lengths up to 135 m. Chromatographic bleed was found to be minimal during temperature programmed chromatography and GC-MS applications. Columns exhibited high durability and capacity, tolerating splitless injection of 0.10 - 0.30 µl samples for up to 6 months before appreciable losses in efficiency occurred.

When used in conjunction with a glass insert designed for splitless injection, large bore glass SCOT columns provided the performance and characteristics necessary for the analysis of sensitive mixtures of biological origin. The inertness of the system should minimize sample losses through adsorption and the formation of artifacts through degradations. The superior column efficiency resulted in high resolution of exceedingly complex mixtures. High column capacity combined with splitless injection provided sufficient sample for GC-MS analysis of trace components and for post-column splitting of column effluents for simultaneous chromatographic detection and organoleptic evaluation.

This work has shown that efficient and thermostable polar-phase glass SCOT columns can be produced by dynamic coating with Silanox. With reasonable care, the technique produces columns having reproducible chromatographic properties. Whether all polar phases can be used may depend on their solubility in the employed mixture.

- B -

MICROREACTOR FOR SUBTRACTION CHROMATOGRAPHY

INTRODUCTION

This section contains a review of the methodology and reagents used for subtraction gas chromatography, a description of the fabrication of a simple microreactor for subtraction gas chromatography, and results obtained with the microreactor for the selective subtraction of alcohols, aldehydes, and ketones from a test mixture.

The following study is an expanded version of a recently published paper on this work by McKeag and Hougen (1977b).

LITERATURE REVIEW

A frequent problem confronting the analytical chemist is the qualitative and quantitative analysis of a mixture of many unknown compounds. A popular approach to this problem is analysis by gas chromatography (GC). A large majority of investigations rely upon chromatographic peak areas for quantitation and retention time for identification. In particular, when mixtures are complex and many compounds are unresolved, identification based on retention data should only be considered to be tentative and quantitation without regard for proper detector response factors should be considered an approximation.

The best methods for peak identification are usually considered to be mass spectrometry (MS), infrared spectroscopy (IR), and other spectroscopy techniques. Where conventional trapping of gas chromotographic peaks for spectral analysis becomes impractical, and the expensive on-line GC-IR or GC-MS instrumentation is not available, various ancillary techniques may be employed for structural elucidation.

Subtraction gas chromatography may be regarded as such an ancillary technique; it provides a rapid method for functional group analysis and can serve as a useful aid in the identification of chromatographic peaks. The general principle of subtraction gas chromatography is that specific types of compounds, for example, alcohols, are removed or "subtracted" from the injected samples. The non-subtracted compounds

pass through the chromatograph unaffected. A popular form of subtraction gas chromatography is on-line precolumn reaction involving either irreversible adsorption or the formation of nonvolatile derivatives of the compounds to be subtracted. Many reagents for the subtraction of compounds containing various functional groups have been reported in books by Leathard and Shurluck (1970) and Ma and Ladas (1976), and in a review by Beroza and Inscoe (1969). Typical reagents for subtraction gas chromatography are listed in Table 2.

TABLE 2. Reagents for subtractive gas chromatography.

Reagent	Substance subtracted	Reference
Molecular sieve 5A	*n*-Alkanes	Adlard and Whitam (1961)
Mercuric acetate-mercuric nitrate-ethylene glycol	Alkenes	Kerr and Trotman-Dickenson (1958)
Molecular sieve 10X	Aromatics	Brunnock and Luke (1969)
Boric acid	Alcohols	Ikeda *et al.* (1964)
o-Dianisidine	Aldehydes	Bierl *et al.* (1968)
Zinc oxide	Acids	*ibid*
Phosphoric acid	Epoxides	*ibid*
Benzidine	Aldehydes plus ketones	*ibid*
Phosphoric acid	Nitrogen bases	Fryčka and Pospíšil (1972)

Various reactor designs have been used to obtain quantitative subtraction of compounds during gas chromatography. The basic design of all reactors consists of a tube containing a reagent through which the vaporized sample passes prior to separation in the analytical column. Reactor designs are listed in Table 3.

TABLE 3. Reactor designs for subtractive gas chromatography.

Reactor	Location in chromatograph	Reference
Metal tube	Oven	Allen (1966)
Metal loop	Oven	Bierl *et al.* (1967)
Glass tube	Injector port	Cronin (1972)
Carbon skeletal apparatus	Injector port	Haken *et al.* (1972)
Spring-loaded metal tube	Injector port	Ladas and Ma (1973)

The subtraction efficiency has been shown to depend on reagent concentration by Prokopenko *et al.* (1972) for the subtraction of alcohols by boric acid and oxygen-containing compounds by lithium aluminum hydride. The dependence of subtraction efficiency upon temperature has been shown by Withers (1972) for the subtraction of aldehydes by FFAP, by Haken *et al.* (1972) for the subtraction of aldehydes and ketones by bisulphite, and by Cronin (1972) for the subtraction of alcohols by 3-nitrophthalic anhydride and carbonyls by semicarbazide.

MATERIALS AND METHODS

Microreactor Construction

The stainless steel microreactor (Figure 6) consisted of a modified union and a reactor tube. The modified union was fabricated from a standard Swagelok union (1/8 in.) joined to a Swagelok nut (1/8 in.) by silver soldering; it was bored through to allow insertion of the reactor tube. The reactor tube was made from a straight piece of tubing (1/8 in. o.d.). A notch was made at one end of the reactor tube to facilitate the flow of carrier gas into the tube when it was placed in position. The tube was inserted from the column oven into the injection port until its notched end touched the septum. The tube was fastened in this position with the modified union and a set of ferrules. With the ferrules thus affixed to the tube, the tube was removed and cut to the appropriate length (6.13 in.) so that its other end butted against the analytical column (1/8 in. o.d.) when the parts were assembled.

The reactor tube was filled with an appropriate reactor material by gentle tapping and plugged with silanized glass wool at both ends. After insertion of the reactor tube in the injection port, the union was tightened and the analytical column was joined to the other end of the union.

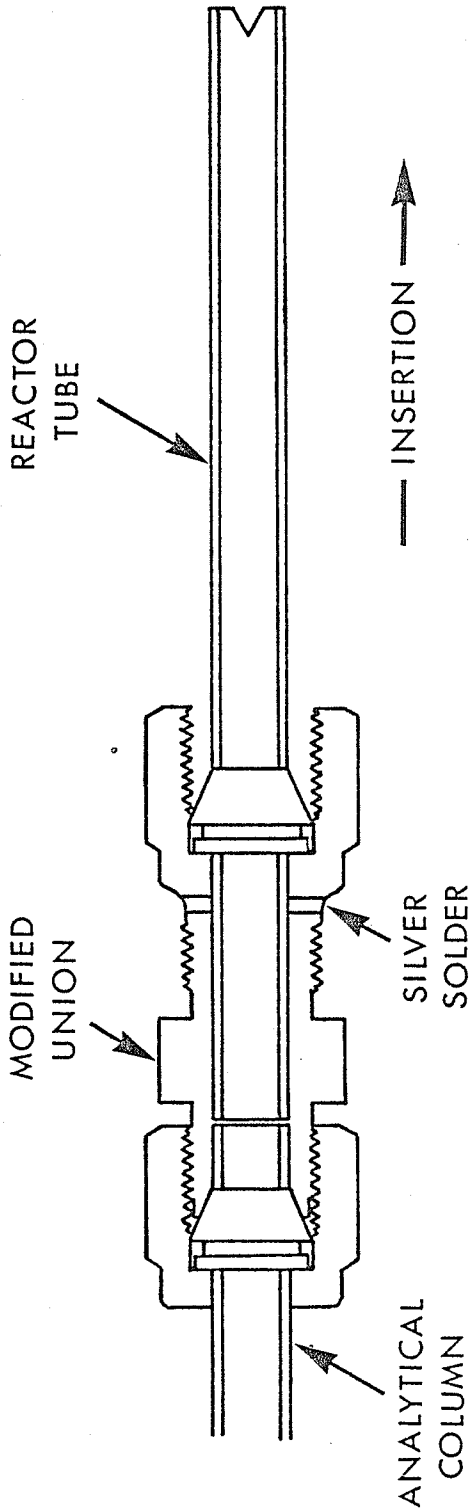


Figure 6. Schematic diagram of microreactor assembly.

Use of the Microreactor

Reactor Materials

Boric acid (Fisher Certified Reagent), finely ground, was mixed with Chromosorb W AW DMCS, 60-80 mesh, in ratios of 5:100 and 8:100 by wt. o-Dianisidine(Baker Practical Grade) was coated from chloroform solution on Wilkens Firebrick (Varian Aerograph Ltd.),and on Chromosorb W AW, both 60-80 mesh and 10:100. Benzidine (Baker Grade) was coated from chloroform solution on Firebrick (60-80 mesh, 25:100), on Chromosorb W AW (60-80 mesh, 30:100), and on Chromosorb W AW DMCS (60-80 mesh, 20:100). Sodium bisulfite (freshly prepared aqueous solution of sodium metabisulfite, BDH reagent) was coated on Chromosorb W AW, and on Chromosorb W AW DMCS, both 60-80 mesh and 15.4:100. Dry sodium bisulfite (by evaporation of aqueous sodium metabisulfite) was mixed in the ratios 5:100 and 10:100 with a coated support material (Carbowax 20M on Anakrom ABS, 70-80 mesh, 1:10).

CAUTION: As noted by Haken *et al.* (1972), benzidine is carcinogenic. The solid and vapor are readily absorbed through the skin. o-Dianisidine should also be treated with caution.

Test Compounds

The compounds used for evaluating the microreactor (Table 4) were selected because of their structural differences, range of boiling points, and availability.

Gas Chromatography

A Varian Aerograph Series 1800 gas chromatograph was used with a flame ionization detector. The microreactor tube was coupled to an analytical column (stainless steel, 8 ft x 1/8 in. o.d.) packed with Carbowax 20M on Chromosorb W AW DMCS, 60-80 mesh, 1:10. The injection

port, i.e., the microreactor tube, was held at a constant temperature which differed with the experiments. The detector temperature was 225°C. The flow of nitrogen carrier gas was 23 ml/min. After injection of each test sample, the column temperature was increased from 60° to 200°C at 4°C/min and held at 200°C until the analysis was complete. Peak areas were measured with an Infotronics Model CRS-100 integrator.

The test compounds (0.1 µl of each), individually or as mixtures, were injected admixed with dodecane (0.1 µl) as an inert internal standard. The amount of a compound subtracted was determined by comparing its peak areas (normalized relative to the internal standard) obtained with and without the microreactor inserted in the instrument.

RESULTS AND DISCUSSION

The microreactor was evaluated for efficiency in subtracting alcohols, aldehydes, and ketones using the reagents boric acid, o-dianisidine, benzidine, and sodium bisulfite. The effects of temperature, reagent concentration, and support materials on reactor efficiencies were examined.

Boric Acid

Boric acid selectively subtracts alcohols from admixtures with other compounds by the formation of nonvolatile borate esters:

$$3\ R\text{-}OH + B\text{-}(OH)_3 \rightarrow (R\text{-}O)_3\text{-}B + 3\ H_2O$$

Boric acid on Chromosorb W AW DMCS was sufficiently reactive at 147°C to quantitatively remove the alcohols tested (Table 4). The aldehydes and ketones passed through the reactor largely unaffected, with retention times increased by about 5% and peak areas reduced by about 2%.

The effect of reactor temperature on the subtraction of 2-propanol and decanol from a test mixture is shown in Figure 7. Although decanol was removed at all temperatures, 2-propanol produced a broad tailing peak at reactor temperatures higher than 147°C, probably from the volatile tri*iso*propyl borate ester formed or from a decomposition product.

Boric acid, mixed with Chromosorb W AW DMCS, 5:100, was an ineffective reagent; all alcohols tested were incompletely removed. Attempts

TABLE 4. Microreactor subtraction efficiency.

Compound	b.p. (°C)	% Subtracted		
		Boric acid[a]	o-Dianisidine[b]	Benzidine[c]
Alcohols				
2-Propanol	82.4	100		
Butanol	117.5	100		
Hexanol	158	100		
2-Octanol	179	100		
Decanol	229	100		
Aldehydes				
2-Methylpropanal	63.5		97.4	100
Pentanal	102.5		100	100
Hexanal	128		100	100
Octanal	168		100	100
Nonanal	185		100	100
Benzaldehyde	178.1		100	100
Ketones				
Propanone	56.5			96.2
3-Pentanone	102.7			100
2-Heptanone	151			100
3-Heptanone	150			100
Acetophenone	202			93.6
2,4-Pentanedione	134			100

[a]On Chromosorb W AW DMCS, 147°C. [b]On Firebrick, 135°C.
[c]On Firebrick, 154°C.

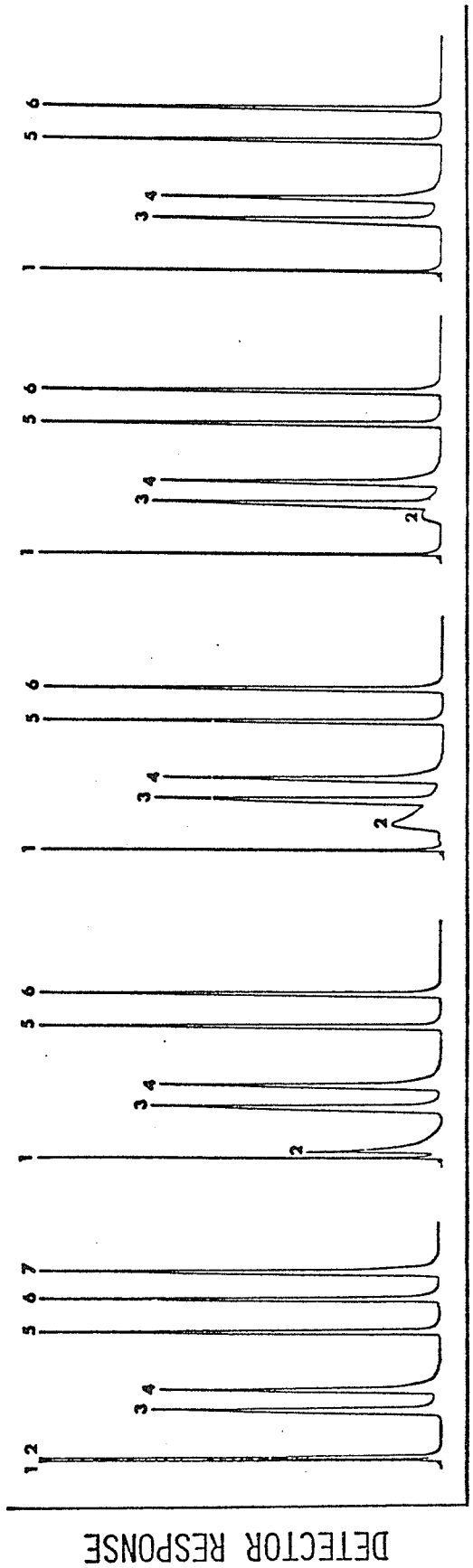
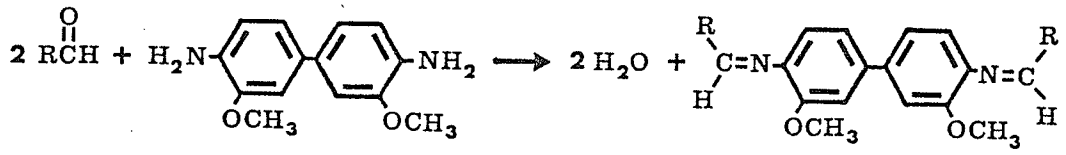


Figure 7. Gas chromatograms illustrating the effects of reactor temperature on the removal of alcohols in a simple test mixture by boric acid. Boric acid on Chromosorb W AW DMCS, 8:100. Peak identity: (1) propanone, (2) 2-propanol, (3) dodecane, (4) octanal, (5) benzaldehyde, (6) acetophenone, (7) decanol.

at obtaining higher reactor efficiencies by lowering the reactor temperature proved impractical; at lower injector (i.e., reactor) temperatures chromatographic tailing became apparent.

o-Dianisidine

o-Dianisidine selectively subtracts aldehydes from admixtures by the formation of nonvolatile Schiff bases:

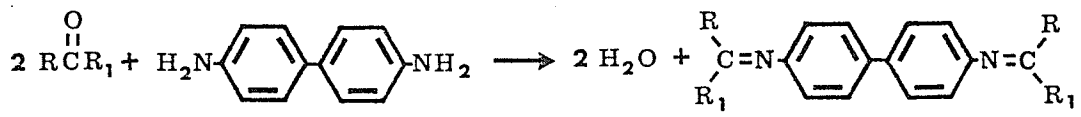


R = alkyl, aryl.

At 135°C, o-dianisidine coated on Firebrick, 10:100, almost completely removed the aldehydes tested (Table 4). The alcohols and ketones passed through the reactor with retention times increased by about 5% and peak areas reduced by less than 6%. Temperatures lower than 135°C reduced the efficiency of the o-dianisidine reagent. No increase in subtraction efficiency was evident at higher reactor temperatures. o-Dianisidine coated on Chromosorb W AW, 10:100, was most efficient at 165°C, removing all the aliphatic aldehydes but only 74% of the benzaldehyde.

Benzidine

Benzidine selectively subtracts aldehydes and ketones from admixtures by the formation of nonvolatile Schiff bases:



R_1 = H, alkyl, aryl.

R = H, alkyl, aryl.

At 154°C, benzidine coated on Firebrick, 25:100, almost completely removed the aldehydes and ketones tested (Table 4). The alcohols passed through the reactor with retention times increased by about 5% and peak areas reduced by about 8%. Benzidine on Chromsorb W AW, and on Chromosorb W AW DMCS, removed the aldehydes but failed to remove the ketones efficiently over a reactor temperature range of 110° to 185°C.

Gas chromatograms illustrating the effects of boric acid, o-dianisidine, and benzidine reagents upon a simple test mixture are shown in Figure 8. The chromatograms clearly illustrate that boric acid has subtracted the alcohols (peaks 2 and 7), o-dianisidine has subtracted the aldehydes (peaks 4 and 5), and benzidine the ketones (peaks 1 and 6) and aldehydes (peaks 4 and 5).

Boric acid was first reported as a subtractive agent for alcohols by Ikeda *et al.* (1964). Bierl *et al.* (1968) first reported the use of o-dianisidine and benzidine, both coated on Chromosorb P, for subtraction of aldehydes, and aldehydes plus ketones, respectively. Haken *et al.* (1972), however, found that benzidine on Chromosorb P (and on Celite and a silanized support) was effective for removing aldehydes but not ketones. Similar negative results were obtained in this work with benzidine coated on Chromosorb W AW and Chromosorb W AW DMCS. The effectiveness of benzidine on Firebrick, for the removal of aldehydes and ketones, may be attributed to the ability of the porous surface of Firebrick to maintain a high reagent load as a uniform coating. Firebrick easily adsorbed 25% benzidine on its porous surface while the same concentration of benzidine on silanized and untreated Chromosorb W formed a thick uneven layer which readily peeled off the support surface

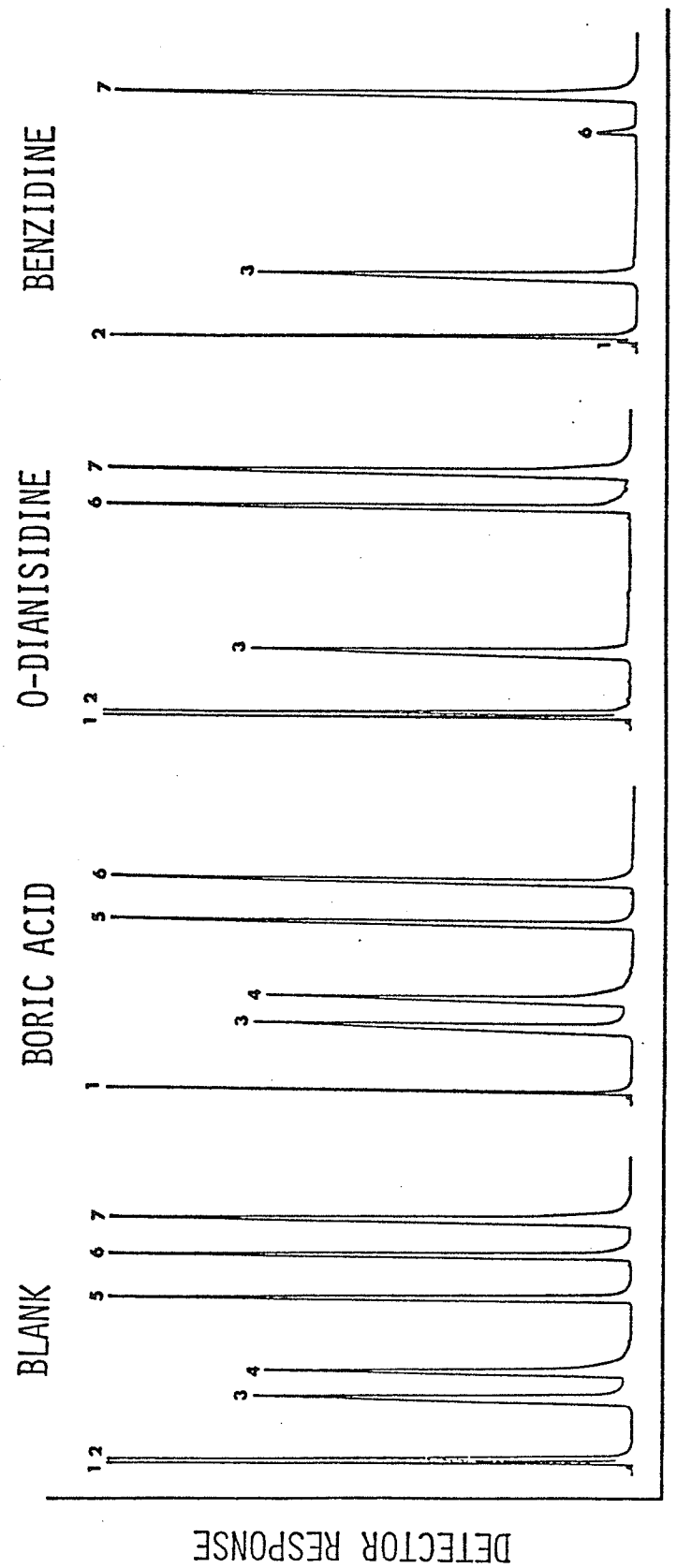


Figure 8. Gas chromatograms illustrating the effects of subtraction reagents on a simple test mixture. Boric acid on Chromosorb W AW DMCS, 8:100, 147°C; 0-dianisidine on Firebrick, 10:100, 135°C; benzidine on Firebrick, 25:100, 154°C. Peak identity: (1) propanone, (2) 2-propanol, (3) dodecane, (4) octanal, (5) benzaldehyde, (6) acetophenone, (7) decanol.

after coating.

Haken *et al.* (1972) have reported quantitative removal of aldehydes and ketones by 11% sodium bisulfite on Celite. In the present work, 15% sodium bisulfite on silanized and on untreated Chromosorb W AW failed to subtract the aldehydes and ketones effectively. The untreated Chromosorb W AW should be similar to Celite in its properties. Decanol, however, was completely removed from the test mixture by the bisulfite reagent coated on Chromosorb W AW, at temperatures of 65^{o} to 115^{o}C. This anomaly may be attributed to a support interaction. The irreversible adsorption of polar substances on a chromatographic support has been reported by Jeltes and Veldink (1968). Sodium bisulfite mixed with the coated support material was equally inefficient; the amounts subtracted generally decreased with increasing reactor temperature from 65^{o} to 165^{o}C. At 65^{o}C the bisulfite reagent coated on or mixed with the coated support material subtracted less than 20% of each aldehyde or ketone peak, with the exception of octanal and nonanal which were 75% removed.

SUMMARY AND CONCLUSIONS

The fabrication of a simple microreactor consisting of a stainless steel reactor tube and column coupling is described. This design may be easily adapted to virtually any gas chromatograph having a heated injection port. Reactor tubes may be easily removed for refilling or substitution of reagents.

The microreactor has been successfully used for the selective removal of alcohols with boric acid, aldehydes with o-dianisidine, and aldehydes plus ketones with benzidine. When used at the optimum temperature for a chosen reagent, the microreactor provided virtually quantitative removal of specific classes of compounds with minimal distortion or delay of unreacted peaks. The reactor capacities were generally sufficient to allow a week of continual daily use before the reagents had to be replaced.

The technique of subtractive gas chromatography provides a simple, rapid method of functional group analysis. Where the concentration of chromatographic peaks is too low for effective trapping for subsequent spectroscopic analysis (e.g., trace components in flavor isolates) and the expensive on-line spectroscopic instrumentation is not available, subtractive techniques may be employed to provide useful information for structural elucidation.

- C -

ODOROUS COMPOUNDS FROM HEATED RAPESEED OIL

INTRODUCTION

This section contains a review of the literature concerning the mechanisms of fatty acid oxidation, the formation of secondary degradation products contributing to off-flavors and off-odors, the degradation products isolated from edible oils and their components, and the flavor- and oxidative stability of rapeseed oil.

The methods used for the isolation, chromatographic separation, chemical identification, and organoleptic evaluation of odor isolate components from heated rapeseed oils and the determination of the extent of oxidative degradation produced by heating and air purging rapeseed oil samples are described and the results discussed.

LITERATURE REVIEW

The effects of heat and oxygen upon lipids have been a subject of intensive investigations in recent years. The mechanism of autoxidation of fatty acids is discussed in the following review with reference to the production of compounds contributing to off-flavors and odors in edible oils.

As the volatile decomposition products from the various edible vegetable oils are predominantly produced from common sources, such as from the naturally occurring fatty acids, a discussion follows on the typical volatile decomposition products produced by heating pure fatty acids and their esters, pure triglycerides, and naturally occurring oils. Special emphasis is placed on the decomposition of the major fatty acids present in rapeseed oil and on the volatile decomposition products known to be responsible for rancidity.

The review terminates with a discussion of the oxidative stability and the off-flavor in heated rapeseed oil.

The Mechanisms of Lipid Oxidation

This section reviews the mechanisms of oxidation of saturated and unsaturated fatty acids and the formation of secondary degradation products.

Saturated Fatty Acids and Their Triglycerides

Analysis of the products obtained by thermal oxidation of saturated fatty acid methyl esters by Ramanathan *et al.* (1959) and saturated fatty acid triglycerides by Endres *et al.* (1962 a,b) led these investigators to conclude that dehydrogenation was the first step in autoxidation of saturated fatty acids.

Crossley *et al.* (1962) isolated *n*-acids and methyl ketones from tricaprin oxidized in air at 190°C. From these products, it was postulated that the dominant attack was on the α-carbon of the triglyceride acids. Further oxidation of the free acids produced resulted in shorter chain length acids. β-Oxidation of the saturated fatty acids of the triglycerides followed by decarboxylation was suggested to explain the formation of methyl ketones.

Studies of Brodnitz (1968) have shown that there is no dehydrogenation involved in the oxidation of saturated fatty acids. The formation of a free radical rather than unsaturation was suggested as the initial step in the autoxidation of saturated fatty acids. In addition, it was reported that saturated fatty acid esters are attacked at all positions on the carbon chain with a preference for attack at the centre of the chain. The latter view has been substantiated by Selke *et al.* (1975) who reported that all methylene carbon atoms of stearic acid, i.e., tristearin, are susceptible to oxidation, with preferential attack at or near the centre of the molecule.

It has been shown by Brodnitz (1968) that saturated fatty acids react with molecular oxygen to produce monohydroperoxides, most probably through a free radical mechanism. Additional studies appear necessary to elucidate the mechanism of reactions in the oxidative break down of saturated fatty acids and their derivatives.

Compared to their unsaturated homologues, saturated fatty acids exhibit high oxidative stability (Badings, 1960). Their oxidation may be accelerated, however, in the presence of peroxides, unsaturated fatty acids, or other potential sources of free radicals (Brodnitz, 1968).

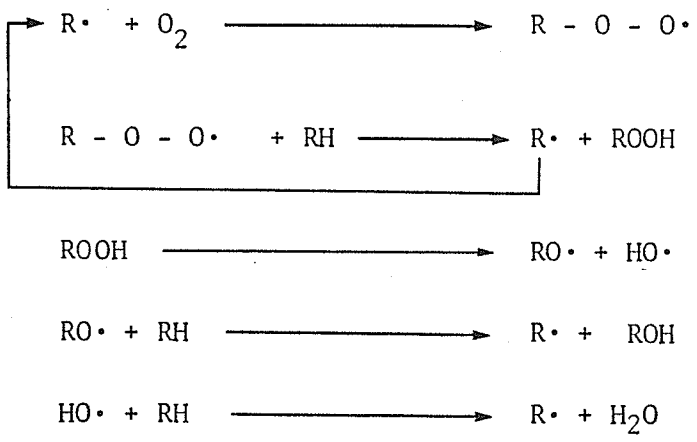
Unsaturated Fatty Acids and Their Triglycerides

It is generally accepted that the autoxidative process for unsaturated fatty acids proceeds by the free radical mechanism postulated by Farmer *et al.* (1942, 1946) and Lundberg *et al.* (1949). The mechanism postulated by these investigators involves a free radical chain-reaction and the formation of hydroperoxides. There are three basic reactions in the autoxidative process:

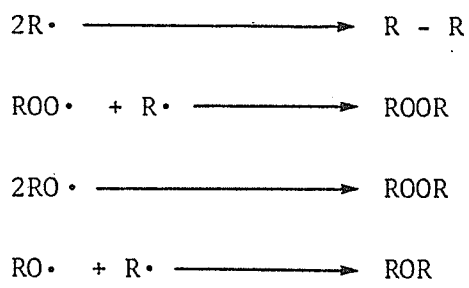
1. Initiation: the formation of free radicals by an initiator such as heat, light, metal, or peroxides.

$$RH \xrightarrow{\text{Initiator}} R\cdot + H\cdot$$

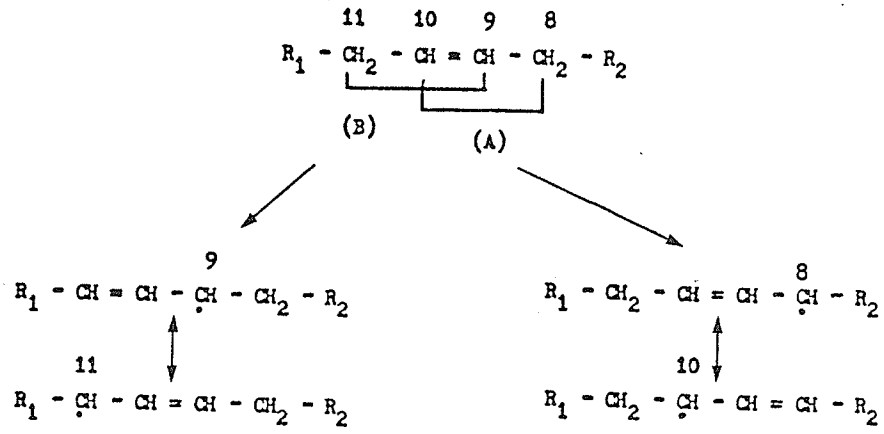
2. Propagation: the free radical chain-reaction with oxygen producing peroxide radicals which in turn react with substrates to generate hydroperoxides and new free radicals. Dismutation of the hydroperoxides leads to the production of off-flavors and additional free radicals for the chain reaction.



3. Termination: the reaction of free radicals to form non-radical products, eg., dimers and polymers.



The methylene groups adjacent to the double bonds of an unsaturated fatty acid are the preferential points of attack in the initiation reaction. For example, the reaction for methyl oleate involves hydrogen abstraction at the allylic positions of the propene systems A and B to produce resonance-stabilized radicals, leading to the formation of four isomeric products as follows:



It has been shown by Privett and Nickel (1959) that the isomeric hydroperoxides derived from the four free radicals illustrated are present in equal amounts in autoxidized methyl oleate. Knight *et al.* (1951) have shown that the autoxidation of methyl oleate induces complete *cis-trans* isomerization of the double bond yielding the more stable *trans* hydroperoxide.

For the unsaturated fatty acids commonly occurring in edible oils the predominant modes of autoxidation can be classified as follows:

1. Monoenoic acids, eg., oleate and erucate: the methylene groups adjacent to the double bond are the preferential points of attack in the free radical reaction, with the formation of four isomeric hydroperoxides.
2. Methylene-interupted dienoic acids, eg., linoleate: the methylene group between the double bonds is the preferential point of attack in the free radical reaction. The formation of three isomeric peroxides is possible.
3. Methylene-interupted trienoic acids, eg., linolenate: the methylene groups between the double bonds are the preferential points of attack in the free radical reaction. The formation of six isomeric peroxides is possible.

In addition, it has been shown by Kharash *et al.* (1953) that at high temperatures, direct attack at the double bond occurs concurrently with the free radical reaction at the adjacent methylene group.

Secondary Products of Autoxidation

The hydroperoxides produced by autoxidation of saturated and unsaturated fatty acids are tasteless and odorless (Henick *et al.*, 1954). The decomposition of hydroperoxides leads to the formation of volatile secondary products responsible for flavor and odor defects in oxidized lipids.

A number of dismutation reactions for hydroperoxides as proposed by Bell *et al.* (1951) are presented in Figure 9.

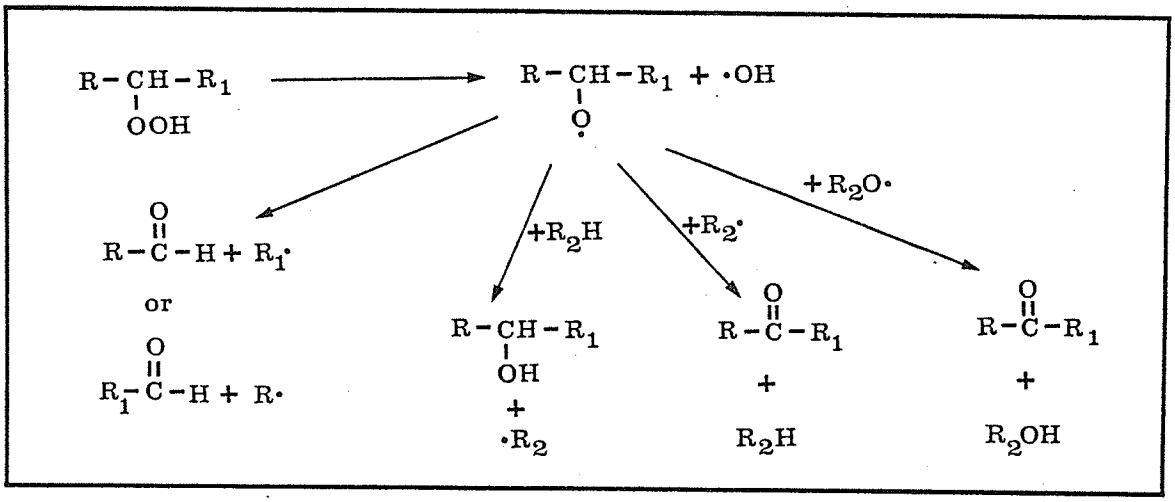


Figure 9. Dismutation of hydroperoxides.

A large number of decomposition products from fatty acid hydroperoxides have been experimentally identified. Badings (1960) has compiled a list of these compounds and compared them with those that have been predicted by theory to be formed. There was excellent agreement between the experimentally observed decomposition products and those predicted by theory.

Volatile Decomposition Products From Fatty Acids and Their Esters

In a study to determine the position of initial attack during the oxidation of saturated fatty acids, Brodnitz *et al.* (1968) oxidized pure methyl palmitate. The major products of oxidation included saturated aldehydes and saturated mono- and dibasic acids. A preference for oxidation at the centre of the palmitate molecule was evidenced by a high proportion of products having 5 to 12 carbon atoms.

Hrdlicka and Pokorny (1962) identified 10 volatile aldehydes as the products of the autoxidation of stearic acid heated for 15 min at 180°C. The oxidation of stearic acid at 100° to 120°C in the presence of a metal catalyst was studied by Paquot and de Goursac (1950). Even numbered acids from C_2 to C_{16}, as well as traces of lactones, were identified.

Skellon and Taylor (1952) have described the autoxidation of erucic acid and of methyl and propyl erucate at 55°, 85°, and 120°C. The oxidative rate, determined by the active oxygen content, was highest for the propyl ester. The oxidation products consisted of small percentages of epoxides, ketohydroxy- and dihydroxy- compounds, aldehydes, and oily complex products. Unsaturated keto derivatives were not conclusively identified.

Gaddis *et al.* (1962) have reported the composition of neutral volatile monocarbonyl compounds from autoxidized esters of oleic, linoleic, and linolenic acids. The esters were oxidized under ultraviolet light at temperatures of 20° and 165° C. The major monocarbonyl products obtained from oleate were C_6, C_7, C_8, C_9, and C_{11} alkanals and C_9, C_{10}, and C_{11} alkenals while the major products obtained from linoleate were pentanal, 2-heptenal and 2,4-decadienal. These aldehydes are those

that would be expected from the decomposition of reported monomeric hydroperoxide isomers. The predominant products from linolenate were 2,4-heptadienal and propanal which suggested that the major monomeric hydroperoxides were the 12- and 16-hydroperoxy conjugated dienoic isomers. The number of minor aldehydes isolated increased with the degree of unsaturation of the fatty acids. Heating at 165°C increased the proportions of the most unsaturated major aldehydes from all fatty acid esters examined.

Hoffman (1962) has reported the isolation of 3-*cis*- and 3-*trans*-hexenal, 2 *trans*-heptenal, 1-octen-3-ol, 2-*trans*-4-*cis*- and 2-*trans*-4-*trans*-heptadienal, hexanal, and nonanal from oxidized methyl linoleate. 1-Octen-3-ol, a compound responsible for reversion flavor in soybean oil was also found in autoxidized rapeseed oil (Hoffman, 1962). Horvat *et al.* (1965) have identified pentanal, hexanal, amyl formate and substituted dioxolanes as the major components of autoxidized methyl linoleate. Minor components included esters, alcohols, ketones, aldehydes, hydrocarbons and acetals.

Kawahara *et al.* (1952) have reported the identification of compounds contributing to reversion of flavor in soybean oil from oxidized linolenate. Hexanal, 1-pentenal, propanal, acetal, and 2-butenal were identified in painty and rancid fractions of odor isolates.

Volatile Decomposition Products from Pure Triglycerides

Crossley *et al.* (1962 b) have reported the thermal oxidation of triglycerides containing lauric, palmitic, and oleic acids in the presence of air at 200°C. The results indicated that long chain carbonyl compounds were formed in the initial stages of the oxidation of saturated fatty acid triglycerides. When the oxidation was allowed to proceed for 24 hr , saturated aldehydes (C_1 to C_6), methyl ketones, and various fatty acids (C_4 to C_{16}) were detected. The presence of dicarboxylic acids

indicated that oxygen attacked the double bond of oleic acid in 1-oleyl dipalmitin.

Selke *et al.* (1975) have reported the identification of 86 volatile decomposition products from tristearin heated in air at 192°C. Methyl ketones and aldehydes were the major degradation products. Minor products included monobasic acids, *n*-hydrocarbons, primary alcohols and γ-lactones. Results indicated that all methylene carbon atoms of the stearic acid molecule were susceptible to oxidation. The quantities of aldehydes and ketones were estimated to be in excess of their flavor thresholds, indicating that even saturated fatty acid triglycerides may be precursors of odors and flavors in lipids.

In a study of 61 volatile components obtained by thermal oxidation of triolein in air at 192°C, Selke *et al.* (1977) have reported seven compounds apparently unique to the oxidation of oleate, namely, heptane, octane, heptanal, octanal, nonanal, 2-decenal, and 2-undecenal. Minor compounds isolated from oxidized triolein included saturated and unsaturated aldehydes, *n*-hydrocarbons, saturated primary alcohols, methyl ketones, γ-lactones, and monobasic acids. Incorporation of stearic acid into the triglyceride molecule appreciably increased the amounts of saturated decomposition products.

The volatile decomposition products of triolein under simulated deep fat frying conditions have been reported by May (1971). Triolein was oxidized at 185°C for 74 hr with periodic injections of steam through the heated triglyceride. Of the 98 components identified, alkanals, alkenals, and acids were the major decomposition products. Some unsaturated γ-lactones were identified and shown by organoleptic testing to be important contributors to the characteristic deep fat fried flavor.

Volatile Decomposition Products From Natural Oils

The isolation of volatile decomposition products from oxidized oils has been the subject of numerous investigations. Of major interest are the identities and sources of two types of odorous compounds, namely, those causing off-odors and the desirable characteristic fried odors.

A comprehensive review of odor and flavor compounds derived from lipids has been compiled by Forss (1972), listing the origins, mechanisms of formation, and threshold values.

The volatile decomposition products from heated vegetable oils originate predominantly from the naturally occurring fatty acids. A summary of studies on various edible oils has been compiled (Table 5). Because the volatile decomposition products of heated triglycerides having mixed fatty acid composition are so numerous, only the major compounds isolated are listed in the Table. The predominance of aldehydic decomposition products is evident. As the threshold values of aldehydes are low, in the parts per million range (Forss, 1972), these compounds may be considered to be a prime source of odors and flavors in oxidized oils.

TABLE 5. Major volatile decomposition products isolated from heated oils.

Source	Treatment	Number of compounds identified	Major compounds	Reference
Corn	185°C Steam	30	C_5-C_9, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, azelaic, 3-decenoic Acids	Kawada *et al.* (1967)
Corn	185°C Steam	65	C_6, C_7, C_9 Alkanals C_7, C_{10} 2-Alkenals C_9, C_{10} 2,4-Dienals	Krishnamurthy *et al.* (1967)
Olive	Steam distillation	77	C_5 - C_7, C_9 Alkanals C_6, C_7, C_{10}, C_{11} 2-Alkenals C_7, C_{10} 2,4-Dienals	Flath *et al.* (1973)
Peanut	180°C Air sweep	15	C_4 - C_7 Alkanals C_7 - C_9 Acids Et-, Pr-, Bu- Benzenes Pentyl alcohol isomers	Fedeli *et al.* (1974)
Soybean	22°C Light	43	C_3, C_5 - C_7 Alkanals C_7, C_{10} 2,4-Dienals C_4 Acid	Selke *et al.* (1970)

The Flavor- and Oxidative Stability of Rapeseed Oil

Traditionally, two major compositional factors have been used to distinguish rapeseed oil and related *Cruciferae* oils from other vegetable oils: the presence of linolenic and erucic acids in the glycerides. Linolenic acid, particularly susceptible to oxidation, has been studied as a possible source of off-flavors in rapeseed and soybean oils (Kawahara *et al.*, 1952). As the type of off-flavor which develops in these two oils is similar, comparisons between the two oils can be fruitful.

Industrial processing has been shown to have a profound effect upon the flavor- and oxidative stability of oils. Appelqvist (1967) has shown the deleterious effects of oxidative conditions during the extraction of oil from rapeseed. Light, and lack of equipment cleanliness, as well as heat damage during seed drying were cited as causes of the development of lipid hydroperoxides.

Kawada *et al.* (1966) have reported that two distinct types of flavors are produced by catalytic hydrogenation of soybean oil. One is the characteristic flavor developed during the hydrogenation process. After this flavor is removed in the deodorization process a storage flavor develops in the previously bland oil. Hydroperoxides were shown to be developed during hydrogenation. The flavors developed in hydrogenated oils were attributed to C_6 - C_9 aldehydes, and ketones and alcohols. The formation of a high percentage of fatty acid *trans*-isomers during selective hydrogenation and the particularly detrimental effects of *trans*-isomer degradation products upon oil quality have been reported by Coenen (1976).

Moser *et al.* (1966) have reported the effects of time and temperature of deodorization upon the stability of soybean oil. Although high temperatures and short times, and low temperatures and long times both produced bland oils, the latter deodorization procedure produced the more stable oil. Ackman *et al.* (1974) have reported that steam deodorization of low-erucic acid rapeseed oils produces linolenic acid isomers. Two major linolenic acid isomers, *cis*-9, *cis*-12, *trans*-15 and *trans*-9, *cis*-12, *cis*-15, as well as two minor isomers, *trans*-9, *cis*-12, *trans*-15 and *cis*-9, *trans*-12, *cis*-15 were detected. The possibility that these linolenic acid isomers can produce unique odorous products should not be ignored.

Holm *et al.* (1957) have established the importance of the degree of oxidation of the oil in the seed on the oxidative stability and the flavor of rapeseed oil, margarine, and cottonseed, peanut, and soybean oils. Aldehyde values (now called benzidine values) and peroxide values revealed that freshly extracted crude rapeseed oil contained oxidation products. A comparison of benzidine values and peroxide values obtained at different stages of refining showed that oxidation products produced during alkali-refining and bleaching could not be removed adequately by deodorization. The analysis of the oxidized products present in refined and deodorized rapeseed oil revealed carbonyl compounds of high molecular weights. The identities of these compounds were not established. There was a negative correlation between the flavor stability of refined oils and their aldehyde contents. Oxidized rapeseed and soybean oils were characterized as oily and green while cottonseed and peanut oils were characterized as nutty, metallic, or bitter.

Moser *et al.* (1965) established a specific flavor effect occurring in *Cruciferae* oils. The flavor-and oxidative stability of oils obtained from crambe seed, mustard seed, rapeseed, and soybean were studied under accelerated storage conditions and after exposure to fluorescent light. Comparisons were made between oils with and without the addition of 0.01% citric acid. The freshly prepared oils had flavors described as buttery, with or without the addition of citric acid. A comparison of the flavor scores obtained with and without the addition of citric acid to the oils showed that citric acid improved the oxidative stability of all the oils stored at 60°C for four days.

The addition of citric acid to soybean oil increased its oxidative stability to the effects of exposure for 2 hr to fluorescent light. This increased flavor stability exhibited by the soybean oil protected with citric acid was not evident in the *Cruciferae* oils. *Cruciferae* oils developed a rubbery flavor which was accompanied by garlic or onion-like notes. It was assumed by the authors that the source of the characteristic sulphurous notes of the *Cruciferae* oils was glucosinolate compounds introduced into the oils from the meal during the extraction process. Apparently, the sulphurous compounds were not completely removed during the deodorization process.

It has been reported by Sattar *et al.* (1976 a) that rapeseed oil was oxidized more rapidly than soybean oil, when exposed to fluorescent light. The more highly oxidized rapeseed oil, however, appeared to better maintain its organoleptic acceptability. These authors have also reported that photochemical oxidation appeared not to be autocatylic, in contrast to the free radical mode of oxidation because samples returned to the dark ceased to be oxidized and because different storage temperatures of

15° and 30°C showed little effect on the oxidation rate (Sattar *et al.*, 1976 b). Photo-inhibitors such as β-carotene present in the oils were suggested as the reason for the different susceptibilities to photooxidation observed for the different oils.

Dobbs and Vaisey (1975) have reported that the odors from heated rapeseed oil were significantly stronger than odors from heated soybean, corn, safflower , and sunflower oils. When rapeseed oil and hydrogenated rapeseed oil were held at 190°C for extended periods of time the odor intensity values and thiobarbituric acid values increased to maximum values in about 10 min. and then levelled off. Although the differences were not significant the hydrogenated oils consistently gave lower odor intensity values than the un-hydrogenated oils. The odor intensity values for low-glucosinolate rapeseed oils were lower and more similar to those of soybean oil than were the values for high-glucosinolate rapeseed oils.

Odors from high- and low- erucic acid rapeseed oil varieties obtained from three Canadian processors were compared with chemical standards for fishy, buttery, sweet, sulphurous, and painty odors (Dobbs and Vaisey, 1975). The high-erucic oils were significantly lower in fishy odors and tended to be lower also in painty and sulphurous odors. The oils obtained from one of the processors were consistently, but not significantly, lower in all odor parameters than those obtained from the other two processors.

MATERIALS AND METHODS

Isolation of Odorous Compounds from Heated Rapeseed Oil

The method used was a modification of the distillation technique of Redshaw *et al.* (1971) for the isolation of volatile material for gas chromatographic analysis. The apparatus used, shown in Figure 10, consisted of an inlet arm for sweep gas, an 850 ml reservoir for the oil under investigation, and an outlet arm connected to a 3 ml capacity cold trap for condensation of the volatile material. The assembly was fabricated in the Chemistry Department Glass Blowing Shop.

The reservoir was placed in the oven of a Varian Aerograph Series 1800 gas chromatograph for dual columns. A 0.125 in. stainless steel tube entering the oven through injector port A of the chromatograph was connected to the gas inlet arm of the reservoir with a 0.125-0.25 in. Swagelok reducing union. The outlet arm was connected to a straight glass tube (3.0 mm o.d., 1.0 mm i.d.) with a ground-glass ball joint secured by a tension clamp. The glass tube extended out of the chromatograph oven through injector port B. The glass cold trap with its 3.0 mm o.d. glass inlet tubing was connected to the tubing extending from the reservoir outlet arm with teflon connectors.

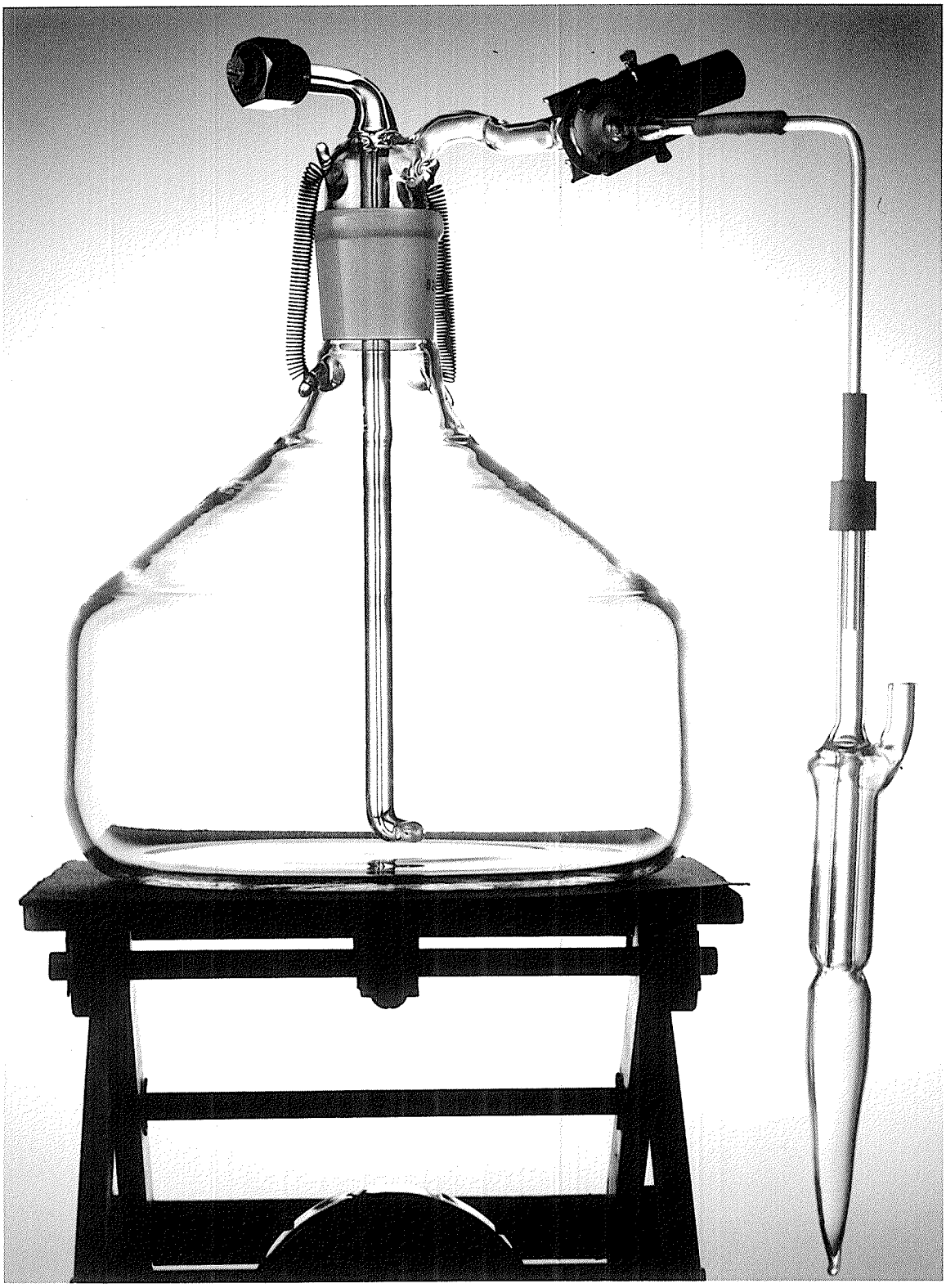


Figure 10. Apparatus for the isolation of volatile components from heated rapeseed oil.

The reservoir was filled with 450 ml of rapeseed oil, placed in the chromatograph oven, and connected to the gas inlet tube and cold trap. Ground glass joints were lubricated with the oil under examination. The chromatograph oven and both injection port temperatures were set at 185°C. Air, filtered through molecular seive 5A, was bubbled through the oil at 40 ml/min for 150 min. The volatile material emanating from the oil was collected in the cold trap maintained at 0°C in ice water.

After weighing the odor isolate in the cold trap, the material was transferred to a cold 2.0 ml vial (Hewlett Packard Part No. 5080-8712), flushed with nitrogen, and capped with a teflon lined cap. The isolate was stored in a freezer at -20°C until analysis, usually 1-2 days.

Volatiles From Commercial Antioxidants

Possible odor contributions of commercial antioxidants present in the oils were determined with the above apparatus by heating an antioxidant mixture in paraffin oil at 190°C for 2.0 hr. A commercial blend of 20% butylated hydroxyanisole (BHA), 20% butylated hydroxytoluene (BHT), and 60% monoglyceride citrate was added to paraffin oil at total antioxidant levels of 0.02%, 0.10% and 0.20%. Filtered air was bubbled through the heated oil at 40 ml/min and the gaseous effluent trapped in the cold trap at 0°C. Vapours issuing from the cold trap during the collection period were monitored periodically by sniffing.

Gas Chromatography of Rapeseed Odor Isolates

Chromatograph Modification

A Varian Aerograph gas chromatograph, model 1800, was used with flame ionization detector. The instrument was modified to permit splitless injection of odour isolates to a glass Support Coated Open Tubular (SCOT) column and to permit optional post column splitting of effluents for simultaneous detection and organoleptic evaluation.

A glass inlet system was fabricated as described in detail in the first section of this thesis, under the heading "Connection of the SCOT Column to the Gas Chromatograph". The methods of splitless connection of the column to the glass inlet and to the detector are described in the same section.

For organoleptic evaluation of chromatographic peaks, a simple effluent splitter was fabricated. A 0.0625 in. Swagelok tee was coupled to the glass SCOT column and to the stainless steel capillary inlet to the flame ionization detector. The side arm of the tee was connected to an L-shaped glass capillary tube (0.74 mm i.d., each arm 30 cm). The column effluent splitter was aligned to exit from the column oven through injector port A of the chromatograph (the "sniffing port").

The split ratio of the column effluent was determined with helium gas and a soap film bubble meter. Measurements were made at the sniffing port and the flame ionization detector flame tip.

Preparation of a Support Coated Open Tubular Glass Column

Glass capillary tubing was extruded according to Desty *et al.* (1960). The capillaries were coated by a dynamic two-step coating process as first described by German and Horning (1973) and modified by McKeag and Hougen (1977 a). Details of the glass capillary extrusion and coating, as well as the conditioning of the coated columns are given in the first section of this thesis under the headings "Glass Tubing Preparation", "Cleaning Glass Tubing", "Extruding Glass Capillary Tubes", "Bending Coiled Glass Capillaries", "Preparation of Support Coated Open Tubular (SCOT) Columns", and "Column Conditioning".

Analytical Separation of Odor Isolates from Heated Rapeseed Oil

Rapeseed oil odor isolates were analysed by splitless injection of 0.10 µl samples to a 135.0 m x 0.62 mm i.d. Silar-5CP glass SCOT column. After injection of the sample, the column temperature was held at 25°C for 10 min , and then increased sequentially at 1°C/min for 99 min, and 2°C/min for 11 min, and held at 146°C until the end of the analysis. The injector and flame ionization detector were maintained at 220° and 240°C, respectively. The gas flow rates were as follows: helium.carrier, 4.0 ml/min; helium make-up gas, 8.0 ml/min; hydrogen, 10.0 ml/min; and air, 100 ml/min. The electrometer attenuation and range were 8×10^{-10} afs.

Peak areas and retention times were measured with an Infotronics model CRS 100 integrator. Chromatograms were obtained from the output of the integrator with a Honeywell Lab/Test model 194 recorder.

Organoleptic Evaluation of Rapeseed Oil Odor Concentrate Peaks

A panel consisting of 6 persons was used, some of whom had prior experience in odor evaluation. As all panel members were familiar with the odors of common laboratory chemicals and with heated oil odors, extensive training was not given. Three standards were presented to the panelists for sniffing and the panelists were asked to describe the odor of the samples with the following terms: hexanal, rancid; pentanoic acid, pungent; and butyl ethanoate, fruity or floral. Panelists were required to sniff the column effluents and to describe the odors of the chromatographic peaks in either chemical or the above descriptive terms and to rate the intensity of the odor on a scale of 1 (weakest) to 7 (strongest).

Chromatographic Parameters

All gas chromatographic parameters in the organoleptic experiments were identical to those described for the analytical separation of the isolates as described in the preceeding section, with the exception of the following: sample size, 0.30 µl; post column split, 6.5:1 (sniffing vent:FID); electrometer attenuation and range, $2x10^{-10}$ afs. Chromatograms were obtained from the output of the chromatograph electrometer, i.e., no electronic integration was used.

Identification of Rapeseed Oil Odor Isolate Components by Gas Chromatography - Mass Spectrometry (GC-MS)

The rapeseed oil odor concentrate was separated with a 135.0 m x 0.62 mm i.d. Silar-5CP glass SCOT column installed in a DuPont Dymaspec GC-MS unit. The instrument was coupled to a Dymaspec 320 Data System. To permit efficient splitless injection of the sample to the SCOT column, the injector port of the gas chromatograph was fitted with a glass injector insert and column coupling unit similar to those previously described.

After injection of 0.10 µl of the odor isolate the oven temperature of the gas chromatograph was maintained at 25°C for 10 min and then increased at 1°C/min for 120 min and maintained at 145°C until the end of the analysis. The injector, jet separator, and source temperatures were maintained at 225°, 225°, and 250° C, respectively. The flow rates of the helium carrier gas and helium make-up gas (added to the column effluent prior to the jet separator) were 4.0 and 16.0 ml/min, respectively.

Column effluents were scanned over a mass range of 50-300 amu using the IFSS scanning option, i.e., the data system selected scan rates to maximize signal/noise ratios for individual peaks. The mass spectral data and total ion currents were stored on a magnetic disc for processing.

After the GC-MS analysis was complete, the data were electronically processed to produce background-free mass spectra and a total ion current chromatogram.

A modified Bieman-type of spectral search was used to compare the obtained mass spectra with those stored in a spectral library. The mass spectra and the results of the spectral search were obtained in printed form.

Fatty Acid Analysis of Rapeseed Oils

Esterification

Rapeseed oils were analysed for fatty acid composition by gas chromatography of fatty acid methyl esters. The esterification procedure used was the rapid methanolysis technique described by Hougen and Bodo (1973). A sample of rapeseed oil (0.85 g) was dissolved in 1.0 ml petroleum ether solution (boiling range 36-57^{o}C, "Skelly F", Skelley Oil Co., Kansas City, Mo.) in a 10 ml volumetric flask with glass stopper and narrow neck (6-9 mm i.d.). One ml of 0.4 N sodium methoxide in absolute methanol was added. The flask was allowed to stand 20 min at room temperature for methanolysis to take place. Water was added, with swirling, until the entire petroleum ether layer rose into the neck of the flask. The layer was allowed to clarify overnight. Samples were withdrawn with a syringe directly from the clear solvent layer for gas chromatographic analysis.

Gas Chromatography of Rapeseed Oil Fatty Acid Methyl Esters

Rapeseed oil fatty acid methyl esters were analysed with the modified Varian Aerograph gas chromatograph and the 135.0 m x 0.62 mm i.d. glass Silar-5CP SCOT column previously described. The column was maintained isothermally at 175^{o}C. The injector and flame ionization detector were maintained at 220^{o} and 240^{o}C, respectively. The gas flow rates were as

follows: helium carrier gas, 4.0 ml/min; helium make-up gas, 8.0 ml/min; hydrogen, 10 ml/min; and air, 100 ml/min. The electrometer attenuation and range were $4x10^{-12}$ afs.

Chromatographic peak areas were measured with a Hewlett Packard 3380A computing integrator used in the area percent mode for computation. The contribution of the solvent peak was electronically rejected using an integration delay of 8 min.

The peak areas for individual fatty acids were statistically analysed using a t-test for the difference between means for triplicate determinations for rapeseed oil samples prior to and after heating with air purging.

Determination of Thiobarbituric Acid (TBA) Values

A modification of the thiobarbituric acid test described by Dobbs (1975) was used. A 0.50 g rapeseed oil sample was added to a 15 ml screw-top glass tube. After the addition of 5.0 ml hexane, the tube was tightly capped and the contents mixed for 15 sec with a vortex mixer. Five ml of aqueous 0.02M 2-thiobarbituric acid (TBA) was added to the tube, the tube was capped and the contents shaken. Tubes were stored overnight in a dark cupboard.

The hexane layer was removed by suction with a pipette. An aliquot of the remaining aqueous solution was transferred with a Pasteur pipette to a cuvette. The absorbance of the solution at 528 nm against a reagent blank was determined with a Coleman Junior Spectrophotometer, Model 6A.

Determination of Hydroperoxide Values

Lipid hydroperoxides were determined using the method of Eskin and Frenkel (1976). An oil sample (0.5 g) was dissolved in 10 ml acetone in a Pyrex centrifuge tube followed by the addition of 0.5 ml titanium reagent (20% $TiCl_4$ in conc. HCl). The solution was mixed thoroughly and the titanium complex formed was precipitated by the addition of 2 ml conc. NH_4OH.

The mixture was then centrifuged at full speed for 5 min on an International Model HN Centrifuge and the supernatant discarded. The precipitate was then redissolved in 4N HNO_3 with the final volume adjusted to 10 ml with the nitric acid and the absorbance was read at 415 nm against a reagent blank.

RESULTS AND DISCUSSION

This chapter is divided into six sections, dealing with the isolation of odorous compounds from heated oils, the gas chromatographic separation of the odor isolates into individual components, the identification of the components by gas chromatography-mass spectrometry, the organoleptic evaluation of the odor isolate components, the effects of heating on the development of oxidative rancidity, and the effect of heating on the fatty acid composition of the oils.

The Isolation of Odor Concentrates from Heated Rapeseed Oils

Five rapeseed oil samples were examined. These samples represented low erucic acid varieties of rapeseed oil. The samples were all finished for the consumer market, i.e., alkali refined, bleached, and deodorized, with commercial antioxidants added. Two of the rapeseed oil samples, referred to as sample numbers 1 and 2, were obtained from a local retail outlet. The varieties of rapeseed from which these samples were obtained are not known. The two samples were produced on different production dates by the same processor. Sample number 3, obtained from a second processor, was produced from Oro seed. Samples 4 and 5 were obtained from a third processor. Sample 4 was produced from Tower seed and sample 5 was hydrogenated Tower oil. For discretion, the identities of the processors are not revealed.

A glass reservoir and trapping system was designed to permit direct isolation of odor isolates, minimizing the possibilities of losses due to irreversible adsorptions and artifact production through catalytic rearrangements associated with metal surfaces. Use of the chromatographic oven permitted accurate temperature control during the heating of the oil samples. A major advantage of this simple direct procedure was that no additional concentration step was required with the isolated material.

The odor concentrates collected from the five rapeseed oils heated to 185°C for 2.5 hr represented about 0.054% of the original weight of the oils. Little difference was noted in the amounts of odor concentrate obtained from the five oil samples examined. The volatile material collected in the cold trap was light brown in color and had a strong aroma, very similar to the odor of heated rapeseed oil.

Preliminary attempts at collecting the volatile material from heated rapeseed oil with the trap immersed in dry ice-acetone proved to be difficult. The delivery tube to the trap became plugged with solidified material. This problem was overcome by immersing the cold trap in ice-water. Some odorous material thereby escaped from the cold trap. Although the recovery of the volatile material cannot be considered to be quantitative, the isolation procedure was reasonably reproducible. As the prime concern in this study was the isolation of volatile material characteristic of heated rapeseed oil odor, the isolation procedure was considered adequate.

No odor was detected by heating and air-purging commercial antioxidant mixtures dispersed in paraffin oil. Antioxidant levels of 0.02%, 0.10%, and 0.20% were used, which represented one, five, and ten times the maximum legal antioxidant levels permitted under present Canadian

regulations. At temperatures of up to 230°C with an antioxidant level of 0.20%, only a slightly acrid odor was detected. This same faint odor was also detected in a paraffin oil blank. No odorous material was obtained in the cold-trap. It was therefore concluded that the commercial antioxidants used in rapeseed oil do not contribute independently to the formation of odors in the heated oil.

Gas Chromatography of Odor Isolates from Heated Rapeseed Oils

Preliminary gas chromatographic separations of odor isolates from heated rapeseed oils revealed that the mixture was exceedingly complex. A fractionation for the separation of olive oil odor concentrates into polar and non-polar fractions with columns containing dry silica gel reported by Flath *et al.* (1973) was attempted with rapeseed oil odor concentrates. Elution of the odor isolates from a silica gel column (Baker No. 3405, 80-200 mesh) with pentane failed to produce the two major bands reported. Similarly, a separation of the rapeseed oil odor isolate was attempted by thin layer chromatography on silica gel plates (Baker-Flex Silica gel 1B, Cat. No. 0-4462) eluted with pentane, but no separation was obtained. As the hydrocarbons were either absent or present only in minute concentration, this pre-gas-chromatographic fractionation procedure was abandoned. It was also a matter of concern that excessive pretreatment might alter the composition of the odor isolate.

Preliminary gas chromatographic separations of the rapeseed oil odor isolates were performed on three glass SCOT columns each coated with a different liquid phase. Separations obtained with a Silar-5CP column were judged to be superior to those obtained with OV-101 and OV-225 columns.

A large bore (0.62 mm i.d.) Silar-5CP column employed in the organoleptic and GC-MS studies was a compromise between high sample capacity and high chromatographic efficiency. The column easily tolerated splitless injection of 0.30 µl samples of odor concentrate, which permitted organoleptic assessment of the odor character of the individual peaks and also provided adequate resolution for mass spectral analysis.

Gas chromatography of the rapeseed oil odor isolates revealed 138 components. All five odor isolates contained the same 138 components. Only minor differences were evident in the concentrations of the component peaks between the four concentrates obtained from the unhydrogenated oils. For the hydrogenated oil sample, however, the total concentration of volatiles in the odor concentrate was about 35% lower than for the other four oils, and the relative amounts of the individual components also differed.

Repeated gas chromatographic separations of the odor isolates revealed a major problem in attempts to obtain a quantitative estimation of the concentrations of the volatile components. The odor concentrate contained some unidentified and presumably highly polar material which was strongly adsorbed on the chromatographic stationary phase. This adsorbed material could not be completely removed by purging the column at 225^{o}C with helium carrier gas for 72 hr. Under these conditions the adsorbed material eluted slowly, producing a slightly raised baseline. With two successive injections of the odor concentrate, the second injection produced a large hump spanning the central portion of the chromatogram, presumably arising from the strongly adsorbed material. Chromatographic peaks appeared superimposed on the surface of the hump. In addition to the technical difficulty of integrating such peaks, the possibility existed that the strongly bound polar material acted as a secondary stationary phase. Under such circumstances, quantitative estimations of the odor concentrate

peaks were considered to be unreliable.

The polar material in the sample had sufficiently long retention time that it did not interfere with organoleptic or GC-MS studies when a freshly conditioned or silanized column was used. Removal of the strongly bound polar material was achieved by injection of Silyl-8 silanizing reagent at the completion of each chromatogram followed by column purging for 12 hr at 200°C.

A gas chromatogram showing the separation of a rapeseed oil odor concentrate into 138 components is shown in Figure 11. The 15 largest peaks shown in the chromatogram constituted about 86% of the total volatile material. This chromatogram may be considered to be typical of all the rapeseed odor isolates examined. The numbered peaks are referred to in the following sections describing results obtained during GC-MS and organoleptic investigations.

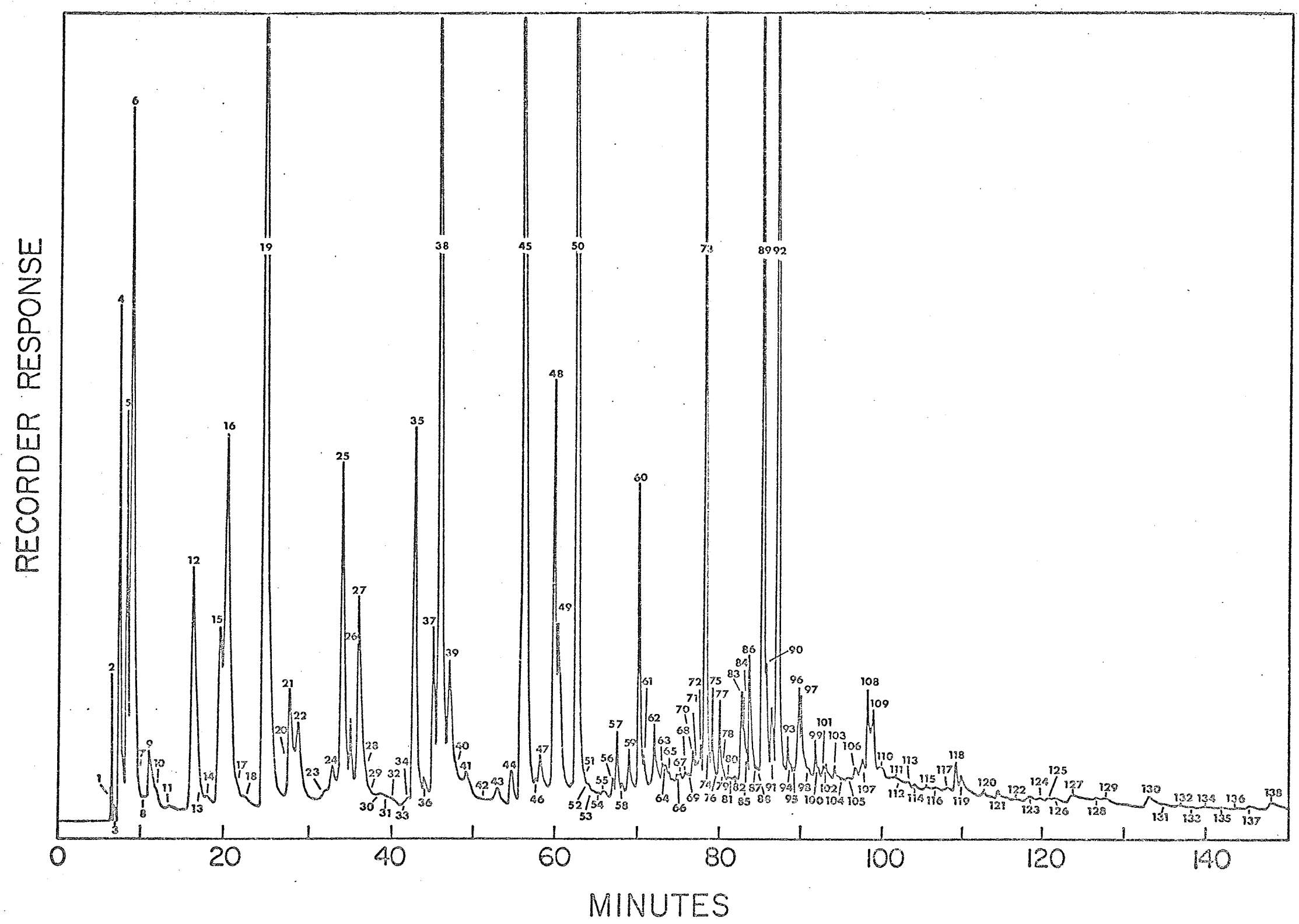


Figure 11. Gas chromatogram of odor isolate from heated rapeseed oil.

Identification of Heated Rapeseed Oil Odor Concentrate Components by Gas Chromatography - Mass Spectrometry (GC-MS)

Two preliminary attempts were made to identify rapeseed oil odor isolate components by GC-MS. A Finnigan 1015 GC-MS unit located in the Chemistry Department of the University of Manitoba was found to lack sufficient sensitivity for identification of capillary column effluents. At the National Research Council of Canada, the Prairie Regional Laboratory, Saskatoon, a Finnigan 1015 GC-MS unit fitted with a large-bore stainless steel tubing inlet to the mass spectrometer also was found unsuitable for analysis of capillary column effluents.

The final GC-MS analysis of the rapeseed odor concentrate was undertaken at the DuPont Company, Instrument Products, Scientific and Process Division, Applications Laboratory, Monrovia,California.

The design of the gas chromatograph oven of the Dymaspec system made it exceedingly difficult to install the Silar-5CP glass SCOT column. Considerable glass blowing and manipulation of fittings was necessary. When installed, the glass insert for splitless injection and the capillary column performed well.

The total ion current chromatogram produced by the data system after the analysis was extremely similar to the conventional FID chromatogram. Trace components were easily visible on the computer trace. This was in part due to the high sensitivity of the mass spectrometer and also to the use of the IFSS scanning mode. In this mode, the computer selects scan rates to maximize the signal/noise ratio.

Identification of compounds from the background-free normalized spectra produced by the computer proved to be exceedingly difficult.

Higher m/e peaks of weak intensity were frequently subtracted by the computer operator, along with the background. In addition, comparison of spectra with literature references often revealed differences in relative peak intensities. Variations in relative peak intensities are produced, however, during rapid scanning mass spectrometry, as noted by Bondarovich *et al.* (1967).

The modified Bieman library search for spectral matching was only marginally useful, possibly because of missing higher mass peaks and spectral library limitations. This search procedure is usually very successful and involves matching the two most intense peaks in every 14-amu interval throughout the entire spectrum. This search technique has been described in a lucid account by Karasek and Michnowicz (1976).

The most successful approach to compound identification in this study was identification of chromatographic peaks by gas chromatographic retention and confirmation of the experimental mass spectra with reference spectra. Using the technique of adding standard compounds for gas chromatographic identification of peaks was moderately successful. More than 100 standard compounds were used for injection with the odor concentrate, usually at levels of 5% of the volume injected.

The compounds identified from the rapeseed odor concentrate are listed in Table 6. Many compounds were not identified, either because they were present in too small amounts, or because their mass spectra were confused due to multiple peak overlap in the gas chromatographic separation. As with any identification scheme based on low resolution mass spectra, many components remained unknown because their mass spectra could not be interpreted.

TABLE 6. Compounds identified from heated rapeseed oil odor concentrate.

Peak no. [a]	Compound	Identified by GC retention	MS data, m/e (relative intensity)
11	Propyl propionate	+	
12	Pentanal	+	57 (60), 58 (100), 71 (15), 86* (6)
19	Hexanal	+	55 (70), 56 (100), 57 (84), 71 (10), 72 (40), 82 (18), 83 (10), 100* (2)
20	2-Hexanone	+	
21	Pentanol	+	55 (100), 56 (12), 57 (36), 88* (0)
25	Heptanal	+	55 (45), 57 (55), 70 (100), 71 (21), 81 (10), 86 (22), 96 (5), 114* (2)
26	2-Heptanone	+	58 (100), 59 (2), 71 (12), 114* (0)
27	*trans*-2-Hexenal	+	55 (100), 57 (60), 69 (52), 70 (21), 83 (64), 97 (79), 98* (15)
30	Ethyl hexanoate	+	83 (100), 99 (48), 60 (45), 73 (47), 71 (15), 101 (24), 61 (25), 144* (1)
33	Hydrocarbon		
35	Octanal	+	55 (78), 56 (100), 57 (82), 68 (38), 69 (40), 81 (25), 84 (20), 128* (0)
36	2-Octanone	+	58 (100), 59 (22), 71 (40), 85 (5), 128* (2)
37	Hydrocarbon		
38[b]	2-Octanol	+	55 (100), 56 (60), 69 (48), 70 (54), 83 (17), 84 (15), 98 (7), 112 (2), 116* (0), 130* (0)
39[b]	Heptanol	+	
43	2,4-Heptadienal		53 (24), 67 (45), 81 (100), 110* (22)
45	Nonanal	+	56 (80), 57 (100), 69 (39), 70 (21), 81 (15), 95 (34), 98 (48), 142* (0)

TABLE 6. cont.

Peak no.	Compound	Identified by GC retention	MS data, m/e (relative intensity)
49	Octanol	+	55 (94), 56 (100), 69 (48), 70 (25), 83 (28), 84 (37), 112 (5), 130* (0)
55	Decanal	+	55 (82), 57 (100), 70 (45), 71 (36), 82 (46), 83 (49), 95 (24), 156* (0)
58	Nonanol	+	55 (100), 56 (98), 69 (58), 70 (25), 83 (84), 84 (21), 98 (29), 144* (0)
60	Hexanoic acid	+	55 (60), 60 (100), 73 (84), 74 (28), 87 (31), 99 (10), 116* (0)
62	4-Keto-2-octenal		55 (100), 56 (71), 81 (38), 83 (75), 109 (34), 111 (27), 112 (15), 140* (10)
70	Octanoic acid	+	55 (64), 60 (100), 73 (81), 84 (10), 85 (15), 101 (8), 144* (0)
89	2,4-Decadienal (isomer)		53 (16), 55 (26), 67 (50), 68 (32), 81 (100), 82 (5), 83 (30), 95 (14), 152* (2)
92	2,4-Decadienal (isomer)		53 (22), 55 (28), 67 (39), 68 (20), 81 (100), 82 (21), 83 (25), 95 (20), 152* (7)
99	Nonanoic acid	+	55 (45), 57 (65), 60 (100), 73 (83), 87 (15), 115 (20), 129 (16), 158* (0)

[a] Refers to Figure 11.
[b] Mixed spectrum containing ions characteristic of both compounds.
* Indicates molecular ion.

Twenty-two compounds were positively identified from their mass spectra and gas chromatographic retention times. Two additional compounds were identified as hydrocarbons; they exhibited the characteristic hydrocarbon spectra of repeated clusters of peaks separated by 14 amu, with decreasing intensities at higher mass values. Two additional compounds were tentatively identified by retention data only, their mass spectra being too weak to be interpreted. These 26 compounds constituted about 54 % of the odor concentrate as determined by chromatographic peak areas.

Two apparently well resolved major components could not be positively identified from their mass spectra. Peak number 50 is probably 2-pentyl furan. The mass spectrum compared quite favourably with a published spectrum. The experimentally determined mass spectrum was as follows: 81 (100%), 53 (28), 82 (20), 67 (24), and 110 (8). The molecular ion at mass 138, however, which should have an intensity of 13% for 2-pentyl furan, was not evident. As the mass spectra of alkyl furans and alkyl dienals have been reported to be nearly identical under rapid scan conditions (Selke *et al.*, 1970), the identity of this compound is speculative. The mass spectrum of peak number 73 (53 (20%), 55 (100), 56 (37), 57 (81), 67 (81), 69 (66), 81 (36), 83 (30), 97 (20), 98 (17), 104 (15), and 127 (5)) could not be interpreted. Possibly, this apparently well resolved peak contained more than one component.

The double bond geometries of the two alkenals and three alkyl dienals identified in this study have not been determined because the mass spectra were considered to be insufficiently precise for accurate designations of *cis* and *trans* configurations. An exception was 2-hexenal, where the *trans* configuration was determined by gas chromatographic comparison with a standard.

Organoleptic Evaluation of Heated Rapeseed Oil Odor Concentrate Components

The glass SCOT column and simple glass splitter used for organoleptic evaluation of the aroma concentrate transmitted clearly distinct chromatographic peak odors. At higher chromatograph temperatures, the sweet background common for metal split systems was noticeably absent with the glass system. Injections of 0.30 µl of odor concentrate with a split of 6.5:1 (sniffing port:FID) produced adequate concentrations of components for organoleptic assessment. Odor responses were easily recorded directly on the chromatographic charts as the detector signals and odor stimuli were almost simultaneous.

Preliminary organoleptic evaluation of the odorous components revealed that peaks occurring after 92 min were virtually odorless. This result was not unexpected as the boiling points of these compounds would be expected to be very high. As the major interest in this study was the characterization of components contributing to off-odor, these high-boiling compounds were not evaluated further.

The determined odor characteristics, odor intensities, and identities of the chromatographic peaks are listed in Table 7, together with the uncorrected retention times. Of the 100 peaks evaluated, 38 were classified as either odorless or too weak to be accurately characterized; 10 were estery or floral, mostly of very low intensity; 6 were acrid, mostly with aldehydic overtones, and three were rubbery-sulphurous, rubbery-oily, and waxy-plastic, respectively. The remaining 43 peaks were classified as oily; 11 with green-beany notes, 6 with fishy notes, and 26 with rancid notes. These 43 oily peaks may be considered the most important in contributing to the off-odor of heated rapeseed oil.

TABLE 7. Organoleptic evaluation and chemical identification of rapeseed oil odor peaks.[a]

Peak number	Retention time (min)	Odor description[b]	Odor intensity[c]	Compound
1	6.6	0	0	
2	6.8	0	0	
3	7.1	0	0	
4	7.5	swt,est	2	
5	8.4	swt,est	3	
6	9.1	0	0	
7	10.3	floral	1	
8	11.1	?	tr	
9	11.8	pun	1	
10	13.4	?	tr	
11	14.3	ald,swt	tr	Propyl propanoate
12	16.3	ald,pun	5	Pentanal
13	17.6	0	0	
14	18.2	sulphur,rubbery	5	
15	19.6	oly	3	
16	20.3	acr,oly	3	
17	22.1	acr,pun	3	
18	23.1	ald	2	
19	24.6	ald,ran,oly	6	Hexanal
20	27.3	?	tr	2-Hexanone
21	27.8	ald	2	Pentanol
22	28.7	ald,pun	3	

TABLE 7. cont.

Peak number	Retention time (min)	Odor description	Odor intensity	Compound
23	31.7	?	tr	
24	32.8	est	2	
25	33.9	grn,ald,est	4	Heptanal
26	35.0	est	2	2-Heptanone
27	35.9	ald,pun,grassy	3	*trans*-2-Hexenal
28	36.8	oly,fishy	3	
29	38.0	ald	3	
30	38.4	ald,ran	1	Ethyl hexanoate
31	39.7	oly	1	
32	40.3	?	tr	
33	41.8	0	0	Hydrocarbon
34	42.1	ald	4	
35	42.9	oly,burnt,ran	5	Octanal
36	44.0	oly,ald,burnt	2	2-Octanone
37	45.1	pun	3	Hydrocarbon
38	45.9	oly,ran	6	2-Octanol
39	47.1	oly,spicy	4	Heptanol
40	48.7	grn,vegetable	3	
41	49.2	?	tr	
42	52.2	?	tr	
43	53.0	grn,ald,oly	2	2,4-Heptadienal
44	54.9	est	2	
45	56.2	ald,oly	7	Nonanal
46	57.8	?	tr	

TABLE 7. cont.

Peak number	Retention time (min)	Odor description	Odor intensity	Compound
47	58.3	est	2	
48	59.9	ran,oly,fishy	6	
49	60.3	oly	2	Octanol
50	62.4	oly,ran, pun	6	
51	63.5	0	0	
52	64.0	grn, vegetable	2	
53	64.7	0	0	
54	65.3	0	0	
55	66.2	grn,oly	2	Decanal
56	67.0	?	0	
57	67.7	grn,oly	2	
58	68.2	swt,est	1	Nonanol
59	69.0	grn,nutty	3	
60	70.1	ran,pun,acr	5	Hexanoic acid
61	70.9	grn,ald	2	
62	72.1	waxy,plastic,oly	2	4-Keto-2-octenal
63	73.1	oly,ran,fishy	3	
64	73.7	grn,beany	3	
65	73.9	0	0	
66	74.8	?	tr	
67	75.4	oly	1	
68	76.0	0	0	
69	76.7	?	tr	
70	76.9	pun,ran,ald	2	Octanoic acid

TABLE 7. cont.

Peak number	Retention time (min)	Odor description	Odor intensity	Compound
71	77.3	0	0	
72	77.8	0	0	
73	78.2	ran,oly	4	
74	79.0	?	2	
75	79.3	0	0	
76	79.8	0	0	
77	80.1	fishy,painty,ran, oly	3	
78	80.5	rubbery,oly	3	
79	80.9	oly	1	
80	81.2	est	1	
81	81.6	0	0	
82	82.0	fishy,oly	2	
83	82.8	oly	tr	
84	83.1	oly	tr	
85	83.3	pun	1	
86	83.9	metallic,oly	2	
87	84.6	pun	1	
88	84.9	0	0	
89	85.3	oly,ran	6	2,4-Decadienal (isomer)
90	85.9	?	tr	
91	86.6	oly	tr	
92	87.1	oly,ran	5	2,4-Decadienal (isomer)
93	88.5	?	tr	

TABLE 7. cont.

Peak number	Retention time (min)	Odor description	Odor intensity	Compound
94	88.8	?	tr	
95	89.2	0	0	
96	89.9	est,swt	2	
97	90.1	fishy,oly	1	
98	91.0	0	0	
99	91.9	grn,vegetable,oly	2	Nonanoic acid
100	92.0	?	tr	
101	92.2			
102	92.9			
103	93.1			
104	94.2			
105	95.2			
106	96.9			
107	97.9			
108	98.2			
109	99.0			
110	99.9			
111	101.8			
112	103.2			
113	104.1			
114	105.8			
115	106.7			
116	107.8			
117	108.5			

TABLE 7. cont.

Peak number	Retention time (min)	Odor description	Odor intensity	Compound
118	109.3			
119	110.0			
120	112.8			
121	114.6			
122	118.5			
123	119.9			
124	120.2			
125	121.0			
126	121.8			
127	124.9			
128	126.3			
129	127.8			
130	133.1			
131	135.0			
132	136.8			
133	138.1			
134	140.0			
135	142.0			
136	143.8			
137	145.4			
138	147.0			

[a] The following abbreviations have been used in the table:
acr: acrid, ald: aldehyde, est: ester, grn: green, oly: oily, pun: pungent
ran: rancid, swt: sweet, tr: trace, 0: no odor, ?: ambiguous.

[b] Odor descriptions ordered according to frequency of response from six panel members.
[c] Mean value for six panel members.

In spite of the different terms used by individual members of the panel to describe particular peaks, odor descriptions were remarkably consistent. For example, the terms oily, greasy, and fatty were often used by different panel members to describe the same peak. Estimates of odor intensity values seemed to vary with individual panel members, some panelists assigning consistently higher values while other members consistently lower values. The relative differences between the individual peaks, however, were assigned in a similar pattern by all the panel members.

In addition to those peaks which were classified as rancid or oily, three particular peaks prompted interest. As the odor character of heated rapeseed oil has been classified as rubbery and sulphurous (Moser *et al.*, 1965) peaks 14 and 78 were of interest. A very weak metallic note detected in peak number 86 was also of interest.

The mass spectra of the sulphurous and rubbery components (peaks 14 and 78) were too weak to be interpreted. It should be noted, however, that of the more than 500 spectral matches made by the computer, not one match was a compound containing sulphur. It has been reported by Daun (1975) that refined and deodorized rapeseed oil contains no detectable sulphur. It is possible that rubbery notes may be produced by fatty acid degradation products such as 1-hexyne (Evans *et al.*, 1971) or 1-hexen-3-ol (Forss, 1972). Whether the rubbery and sulphurous notes present in the odors of heated rapeseed oil are truly due to sulphur warrants further investigation.

The major components identified from the odor concentrate were aldehydes. The aldehydes identified constituted 29.6% of the odor concentrate as measured by chromatographic peak areas. Aldehydes are typical products of triglyceride oxidation and their influence on off-

flavors and odors has been well documented. The odor properties and presumed direct precursors of many of the identified components are listed in Table 8.

The reported odor characteristics in Table 8 are similar to those made by the panelists in the present work (Table 7). Furthermore, it appears that a major proportion of the odorous components identified from heated rapeseed oil originate from the unsaturated fatty acids with one, two, and three double bonds (oleic, linoleic, and linolenic, respectively). Although on the basis of this work no definite conclusion can be drawn as to the origin of the unpleasant odors of heated rapeseed oil, the experimental results indicate that the unsaturated fatty acids of the oil may play a major role as precursors of the off-odors. One would further expect that in rapeseed oil the relatively abundant linolenic acid would be the more important precursor, as this fatty acid is known to undergo oxidative degradation at a considerably higher rate than the less unsaturated linoleic and oleic acids (Badings, 1960).

TABLE 8. Organoleptic data[a] and the presumed origin[a] of compounds identified from the odor concentrate from heated rapeseed oil.

Substance	Threshold[b] value (ppm)	Odor	Presumed origin	
			Parent ester	Probable precursor
Propyl propionate	?			
Pentanal	0.07	pungent	$C_{18:2}$	14-OOH $\Delta^{9,12}$
Hexanal	0.15	green	$C_{18:2}$	13-OOH $\Delta^{9,12}$
2-Hexanone	0.25-0.50			
Pentanol	0.5[c]		$C_{18:2}$	13-OOH $\Delta^{9,11}$
Heptanal	0.005	putty	$C_{18:1}$	9-OOH Δ^{10}
2-Heptanone	0.7[c]	blue cheese		
trans-2-Hexenal	0.6	green	$C_{18:3}$	13-OOH $\Delta^{9,11,15}$
Ethyl hexanoate	0.075[c]			
Octanal	0.040	fatty	$C_{18:1}$	11-OOH Δ^{9}
2-Octanone	0.25-0.5[c]			
2-Octanol	?			
Heptanol	0.52		$C_{18:1}$	11-OOH Δ^{9}
trans-2, *trans*-4-Heptadienal	0.10		$C_{18:3}$	12-OOH $\Delta^{9,13,15}$
Nonanal	0.20	tallowy	$C_{18:1}$	10-OOH Δ^{8}
Octanol	?		$C_{18:1}$	10-OOH Δ^{8}
Decanal	0.70	orange peel	$C_{18:1}$	?
Nonanol	0.086[d]			
Hexanoic acid	14[c]			
4-Keto-2-octenal	?		$C_{18:2}$	11-OOH $\Delta^{9,12}$
Octanoic acid	?			
trans-2,*trans*-4-Decadienal	0.020	deep fried	$C_{18:2}$	9-OOH $\Delta^{10,12}$

TABLE 8. cont.

Substance	Threshold[b] value (ppm)	Odor	Presumed Origin	
			Parent ester	Probable precursor
trans-2,*cis*-4-Decadienal	0.020	sweet aldehyde	$C_{18:2}$	9-OOH $\Delta^{10,12}$
Nonanoic acid	?			

[a]Reference: Forss (1972). [b]Threshold medium paraffin oil, [c]milk, and [d]water.

Estimation of the Degree of Oxidation of Heated Rapeseed Oils

The five commercially refined and deodorized rapeseed oils were tested for the presence of oxidation products before and after heating the samples with air purging for 2.5 hr. The results, shown in Table 9, revealed that a measurable amount of preoxidation had taken place in the oils before they were heated. Hydroperoxides, the direct precursors of volatile off-flavors, were detected, as were further breakdown products (detected by the TBA reagent).

The small degree of preoxidation detected by the TBA and hydroperoxide values was not unexpected; the tests are very sensitive, and a small amount of oxidation invariably takes place in all commercial fats and oils under normal storage and handling conditions. It has been reported by Holm *et al.* (1957) that for laboratory refined and deodorized rapeseed, peanut, and soybean oils, the oxidation values as measured by the benzidine test were almost identical with those determined by the peroxide test. The present work showed this general similarity between the TBA values and hydroperoxide values obtained at zero hr (before heating). It was further indicated that rapeseed oil was oxidized also in the seed, and that only 85% of the oxidation products could be removed during refining.

TABLE 9. Oxidation values for rapeseed oil before (0 hr) and after (2.5 hr) heating.[a]

Sample	TBA value[b]		Hydroperoxide value[b]	
	Before heating	After heating	Before heating	After heating
1	0.02	0.66	0.01	0.06
2	0.01	0.64	0.02	0.09
3	0.19	0.69	0.11	0.09
4	0.01	0.73	0.04	0.13
5	0.02	0.03	*	*

[a]The oils were heated to 185 C for 2.5 hr with air purging.
[b]Absorbance units.
*Hydroperoxide value not measured.

The data in Table 9 show that extensive oxidative deterioration occurred during the heating of the oils. Two pecularities in the data shown in Table 9 are worthy of note. The high preoxidation state of sample 3 seemed to have little influence upon the final state of the oil as determined by the tests. Sample 5, a hydrogenated oil, developed a high odor intensity during heating which was only slightly lower than the other oils. This was not reflected by the relatively low TBA value measured. The failure of the TBA test to predict odor development in hydrogenated oils has been reported by Dobbs and Vaisey (1975).

Fatty Acid Analysis of Rapeseed Oils

The rapeseed oil samples were analysed to determine if the heating produced detectable changes in the fatty acid composition of the oils. Focus was placed on the concentration of the C_{18} fatty acids. As the relative oxidation rates of oleic, linoleic, and linolenic acid triglycerides have been reported by Badings (1960) to be 1:27:96, particular emphasis was placed on the quantitative estimation of these acids and their isomers. A typical

chromatogram showing the separation of the fatty acid methyl esters is shown in Figure 12. It has been reported by Ackman *et al.* (1974) that the two *trans*-isomers of linolenic acid are produced during the deodorization of the oil. In order to resolve the C_{18} fatty acid isomers, the Silar-5CP glass SCOT column was maintained isothermally at 175°C.

The fatty acid compositions of the oils are listed in Table 10. Only the major esters up to C_{20} are listed. Higher esters eluted as broad peaks which could not be quantitated.

TABLE 10. Fatty acid compositions of rapeseed oils.

Fatty acid[a]	Fatty acid composition (area percent)[b]				
	Sample number				
	1	2	3	4	5
$C_{16:0}$	3.77 *3.85*	3.74 *3.96*	4.70 *4.80*	4.66 *4.82*	4.97 *5.09*
$C_{18:0}$	1.57 *1.65*	1.62 *1.65*	2.50 *2.26*	1.81 *1.99*	19.7 *19.9*
$C_{18:1}$	59.5 *61.9*	58.5 *60.6*	62.5 *63.9*	59.1 *60.8*	66.4 *66.5*
$C_{18:2}$	21.1 *19.5*	21.7 *20.5*	19.3 *18.5*	21.8 *20.0*	2.51 *2.17*
$C_{18:3}$ (c,c,t)	0.819 *0.811*	0.771 *0.742*	0.537 *0.539*	0.320 *0.328*	0.020 *0.020*
$C_{18:3}$ (c,c,c)	8.43 *7.79*	8.60 *8.18*	5.38 *5.16*	7.25 *6.99*	0.104 *0.076*
$C_{18:3}$ (t,c,c)	1.22 *1.00*	1.13 *0.931*	0.740 *0.712*	0.478 *0.464*	0.257 *0.228*
$C_{20:0}$	0.525 *0.512*	0.546 *0.480*	0.670 *0.656*	0.658 *0.666*	1.12 *1.20*
$C_{20:1}$	2.39 *2.29*	2.22 *2.08*	1.69 *1.57*	2.40 *2.34*	2.38 *2.20*
Other esters	0.71 *0.67*	0.86 *0.88*	1.98 *1.92*	1.57 *1.65*	2.54 *2.66*

[a]The linolenic ester isomers are those identified in Figure 12.

[b]The mean of triplicate determinations: before heating, boldface type; after heating, italicized type.

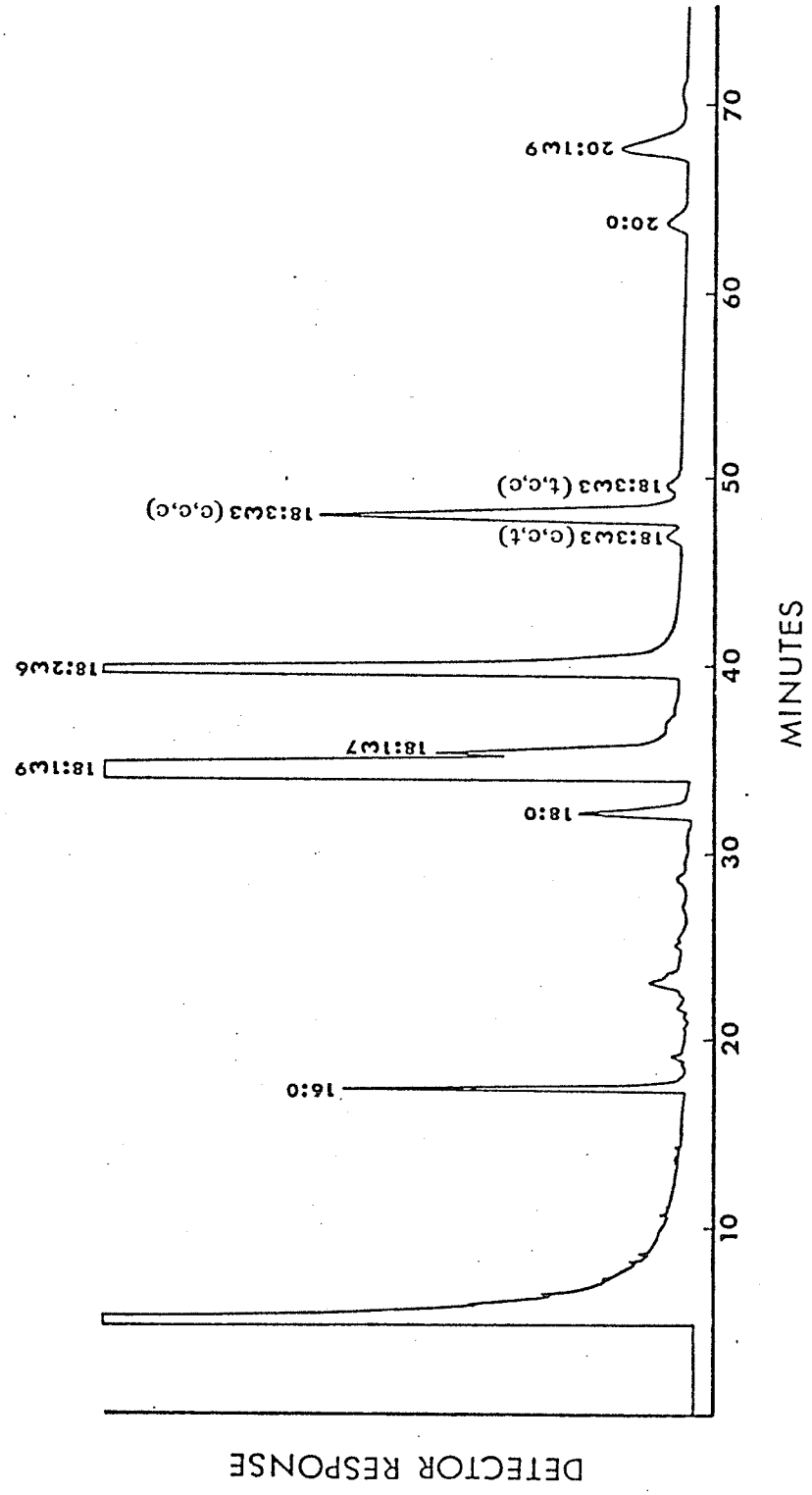


Figure 12. Gas chromatogram of rapeseed oil fatty acid methyl esters. The *cis* and *trans* ethylenic unsaturation in the 9, 12, and 15 positions of the linolenate isomers are denoted in that order by c and t respectively.

The fatty acid compositions before and after heating showed no statistically significant differences. This result was not unexpected as the amounts of odorous material isolated from the oils were very small. It has been reported by Kurkela and Petro-Turza (1975) that simulated frying with rapeseed oil produced changes in the C_{18} fatty acid content of the oil and that this effect was retarded by the addition of wheat germ oil. The long frying times of up to 88 hr used in their study could account for the changes noted.

SUMMARY AND CONCLUSIONS

The odor isolate collected from heated rapeseed oil during heating to 185°C for 2.5 hr represented 0.054% by weight of the origional oil. Gas chromatographic analysis revealed that all five samples tested contained the same 138 components. The 15 largest components constituted 86% of the odor concentrate.

Organoleptic evaluation of the chromatographic peaks from the rapeseed oil odor concentrate revealed a number of "oily" and "rancid" components which presumably are the major contributors to the off-odors of the heated oils.

The twenty-four compounds identified in this study were the first compounds reported in the literature to be responsible for the odor of heated rapeseed oil. However, an unpublished study of the odorous components from heated rapeseed oil by Fedeli (E. Fedeli, Experimental Institute for the Fats and Oils Industries, Milan, Italy; personal communication) has reported 25 compounds, 6 of which have been reported in the present study. In the present study, 11 aldehydes, 5 alcohols, 3 acids, 3 methyl ketones, and 2 esters were identified. In addition, two compounds were established to be hydrocarbons but their structures were not fully elucidated. The 26 compounds and the 11 aldehydes represented 54% and 29.6% of the odor concentrate, respectively.

The major proportion of the identified odor components are presumably generated by oxidative degradation of the unsaturated fatty acids. These acids, linolenic acid in particular, are therefore believed to be the major precursors of the off-odors in heated rapeseed oil. It is of interest to note that the two oils commonly associated with flavor instability, namely,

rapeseed oil and soybean oil, are both exceptionally high in linolenic acid content.

An assessment of the odor contribution of a commercial antioxidant mixture showed that antioxidants did not contribute to the odor of the heated oil.

The five commercial rapeseed oil showed a slight degree of oxidation prior to heating, as measured by hydroperoxide and thiobarbituric acid values. Heating of the oils with air purging produced sufficient degradation of the oils to be detected as off-odors but not as changes in the fatty acid composition.

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