Derivative Formation in the Chromatographic Analysis of Food Additives

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Abstract

The use of derivatization in the analysis of food additives by chromatographic means has been reviewed. The large majority of these derivatization techniques are employed to facilitate the gas-liquid chromatographic quantitation of various food additives. Silylation, alkylation, acylation, as well as transesterification, saponification, and chemical decomposition techniques are discussed. The review includes procedures for the analysis of emulsifiers and stabilizers, artificial sweeteners, antioxidants, preservatives, gums, and waxes, and draws in part, from work conducted in the authors' laboratory.

Introduction

Since its inception over 25 years ago, gas-liquid chromatography (GLC) has probably become the most widely used analytical technique for the analysis of food additives (1). Although some food additives can be analyzed directly by GLC, many others contain polar functional groups or are of high molecular weight and are therefore not suitable for direct GLC analysis. In these cases, chemical derivatization is employed to impart the necessary thermal stability, volatility, and desirable chromatographic behavior so that these additives may be analyzed by GLC. More recently, high performance liquid chromatographic (HPLC) techniques for food additives have been gaining in popularity. However, these techniques have been applied mainly to underivatized compounds, especially those possessing a UV chromophore, and therefore, are not included in the present review. It is conceivable that the use of derivatization in HPLC to impart detectability to non-UV absorbing compounds may receive more attention in the future (2).

Thus, the present review, which includes much of the work conducted in the authors' laboratory over the past decade, will be mainly devoted to GLC derivatization techniques. No attempt will be made to cover the multitude of individual food additives which have been analyzed in a derivatized form: e.g., monosodium glutamate (3), ethylenediamine tetraacetate (4), sodium nitrate (5), although for some of these, in particular monosodium glutamate and amino acids in general, considerable attention has been devoted to the search for suitable derivatives (3). Instead, the derivatization techniques will be illustrated by reference to some of the more important classes of additives such as those listed below:

(A) Emulsifiers and stabilizers
(B) Artificial sweeteners
(C) Antioxidants
(D) Preservatives
(E) Gums
(F) Waxes

The term derivatization in this review includes any chemical reaction (or reactions) which modify a molecule to make it more suitable for subsequent chromatographic analysis, but does not include post-chromatographic derivatization techniques such as those employed after paper, thin-layer, or high performance liquid chromatographic separations.

Emulsifiers and Stabilizers

Emulsifiers

As far as amount produced and consumed is concerned, emulsifiers represent one of the most important classes of food additives. The most common emulsifiers are the mono- and diglycerides which are compounds of glycerol and fatty acids. However, other polyols such as polyglycerol, sorбитol, propylene glycol, and sucrose, and other acids such as lactic and tartaric are used to produce emulsifiers possessing more desirable characteristics. Examples of some of the emulsifiers in current use are shown in Table I.

<table>
<thead>
<tr>
<th>Table I. Examples of Emulsifiers Used in Fatty Foods</th>
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<tbody>
<tr>
<td>Mono- and diglycerides</td>
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<tr>
<td>Propylene glycol fatty acid esters</td>
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<tr>
<td>Sorbitan fatty acid esters</td>
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<tr>
<td>Sucrose fatty acid esters</td>
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<tr>
<td>Polyglycerol fatty acid esters</td>
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<tr>
<td>Ethoxylated monoglycerides</td>
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<tr>
<td>Ethoxylated sorbitan fatty acid esters</td>
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<td>Lactylic esters of fatty acids and their salts</td>
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The complexity of these compounds plus their variability in composition from manufacturer to manufacturer presents considerable problems to the analyst faced with the task of determining small amounts of these compounds, often in admixture with other emulsifiers, in foods. These problems have been discussed in several reviews (6-8).

In the analysis of such compounds the advent of gas-liquid chromatography (GLC) and the availability and use of various derivatizing agents have played an important role.

Transesterification. This technique, using either acid- or base-catalyzed alcoholysis to yield fatty acid esters (usually methyl esters) which are analyzed by GLC, represents the most common derivatization technique available for emulsifier analysis. Although this technique may be useful for quality control purposes to ensure a constant fatty acid content in the emulsifier itself, it has limited use in determining emulsifiers in...
In general, if the emulsifier is known and if a satisfactory separation of the emulsifier can be obtained from various other food constituents, including other food emulsifiers, methyl ester formation and GLC analysis with an appropriate internal standard can provide a satisfactory quantitative determination.

Saponification. Instead of determining the fatty acid part of the emulsifier, attempts have also been made to determine the emulsifier via the polyol moiety. For instance, a method for the analysis of sorbitan monostearate was proposed by Wetterau, et al. (11) based on a constant proportion of isosorbide being obtained on saponification (the isosorbide was determined by GLC). However, subsequent studies by Murphy and Grisley (12) showed the yield varied from supplier to supplier, and therefore, for quantitative determination the source had to be known.

Acetylation. The ethylene oxide adducts of fatty alcohols have been determined by GLC of their acetate esters. Adducts containing up to 13 units of ethylene oxide could be satisfactorily analyzed (13). Free polyglycols in polyethoxylated stearic acid were also determined as their acetates on a 2 ft 20% SE-30 column with diphenyl methane as internal standard (14). Monoglycerides were also chromatographed successfully as their acetyl derivatives (15). The use of these derivatives was largely superceded, however, by the introduction of the trimethylsilyl ether (TMS) derivatives.

Trimethylsilylation. Undoubtedly one of the most important advances in the analysis of emulsifiers was the demonstration that the TMS derivatives of many of these compounds could be conveniently prepared and chromatographed (16). This allowed the emulsifier to be analyzed intact. Sahasrabudhe's (and co-workers) (17) work in this area deserves special mention. Employing an initial fractionation into lipid classes by silicic acid column chromatography, Sahasrabudhe and co-workers developed GLC procedures for the characterization and analysis of the TMS derivatives of mono- and diglycerides (18), polyglycerol fatty acid esters (19), propylene glycol fatty acid esters (20), and the sorbitan fatty acid esters (21). The TMS derivatization conditions were simple: 30-50 mg sample dissolved in 0.5 ml pyridine was treated with 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane, shaken 15-30 seconds, allowed to stand 5 minutes, and analyzed by GLC.

The direct GLC determination of glycerol and mono- and diglycerides as their TMS derivatives in emulsifiers, shortenings, and vegetable oils was examined by Blum and Koehler (22). With no prior separatory steps they analyzed the derivatized compounds directly on 1 ft x 4 mm i.d. glass columns packed with 3% OV-1. In the case of emulsifiers themselves and shortenings where the emulsifier content was high, the method proved satisfactory. Where the emulsifier content was <2%, as in vegetable oils, interferences were observed.

The ethylene oxide adducts of fatty alcohols were also analyzed as their TMS derivatives. Adducts with as many as 15 ethylene oxide units were detectable (23).

Chemical Decomposition Techniques. Lee and Putnam (24) determined the chain-length distribution in fatty alcohol polyethoxylates by cleavage of the ether linkages with hydriodic acid and subsequent GLC analysis of the alkyl iodides. Problems arose, however, from reaction of the hydriodic acid with double bonds.

More recently Tsuji and Konishi (25,26) have investigated the use of the mixed anhydrides of acetic and p-toluene sulfonic acids as a reagent for cleavage of ether linkages. This provides for a simultaneous determination of the hydrophobic and hydrophilic groups. For instance, in the case of the fatty alcohol ethoxylates, the fatty alcohol is converted to the acetate and the polyoxyethylene moiety to the ethylene glycol diacetate. With the ethoxylated fatty acids, glycerol and sorbitol fatty acids, etc., the generated alcohol acetates and free fatty acids were determined by GLC on columns packed with FFAP.

Stabilizers
Brominated vegetable oils (BVO), which are formed by the addition of bromine to common vegetable oils such as olive, sesame, corn, cottonseed, and soybean, and sucrose diacetate hexaisobutyrate (SAIB) are widely used in citrus-based soft
drinks in North America as dispersants and stabilizers for the citrus flavoring oils. In Canada, both BVO and SAIB are currently permitted at specific levels (15 ppm and 50 ppm, respectively) in beverages and consequently, methodology for their analysis is important.

**Transesterification.** With BVO, acid-catalyzed transesterification yields methyl esters containing the bromine groups still intact, specifically, methyl dibromostearate and methyl tetrabromostearate. Unfortunately, when examined under a variety of GLC conditions, these esters, particularly the tetrabromo derivative, were prone to decomposition (27). This was resolved by using an anhydrous base-catalyzed transesterification which yielded brominated esters more stable to GLC conditions. During this process the dibromostearate was converted to the corresponding vinyl bromide and the tetrabromostearate to the divinyl bromide and other rearrangement products. Fortunately, this was a reproducible conversion and yielded a quantitative method (27,28). Typical chromatograms of the dibromostearate and tetrabromostearate derivatives resulting from acid- and base-catalyzed methanolysis are illustrated in Figure 2.

SAIB, which can be analyzed directly by GLC (29), can also be analyzed by a procedure based on estimation of the decyl acetate and isobutyrate produced by transesterification with sulfuric acid-decanol. Hexanoic acid was used as internal standard (30).

**Artificial Sweeteners**

The ban of cyclamates in many countries in 1970, and the more recent demonstration of the carcinogenicity of saccharin leaves few, if any, alternative artificial sweeteners. The present review, however, will include both cyclamates and saccharin, as well as the other sweeteners listed in Table II. The polyhydric alcohols have been included as they are added to diabetic or dietetic foods, although the term "artificial" is inappropriate. A review of the analytical methods available for the estimation of artificial sweeteners in foods has recently appeared (31).

**Methylation.** Groebel (32) methylated saccharin using diazomethane and chromatographed the methylated derivative on a 20% SE-30 column using ethyl p-methoxybenzoate as internal standard. This technique was extended by Conacher and O'Brien (33) who used the same derivative of saccharin for its determination in soft drinks but with stearic acid as internal standard. Two peaks were shown to result from this methylation (33), the N-Me and the O-Me derivatives in a constant ratio of 17:3. This was considered to add more specificity to the determination. Saccharin has also been converted to its N-Me derivative using methyl iodide in dimethyl sulfoxide (34), N,N-dimethylformamide dimethylacetal (35), or dimethylsulfate in acetone (36). Any advantages of these techniques (34-36) over the simpler diazomethane technique do not appear to outweigh the disadvantages of longer reaction times and extra extraction steps. The N-Me derivative can be analyzed with a flame ionization detector (FID), or for more sensitive determination, with an electron capture detector (ECD). Diazomethane has also been used to methylate cyclamic acid (37) and can provide a simultaneous determination of cyclamate and saccharin in soft drinks.

A quantitative determination of glycyrrhizin based on diazomethane methylation of its aglycone, glycyrrhetinic acid (produced on hydrolysis), to form its methyl ester has been described (38).

**Acylation.** The most common acylation technique used with this category of food additives is acetylation, which has been

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**Table II. Artificial Sweeteners**

<table>
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<th>Sweetener</th>
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<tr>
<td>Cyclamate</td>
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<tr>
<td>Saccharin</td>
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<tr>
<td>Aspartame</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
</tr>
<tr>
<td>Polyhydric Alcohols</td>
</tr>
<tr>
<td>Sorbitol</td>
</tr>
<tr>
<td>Mannitol</td>
</tr>
<tr>
<td>Xylitol</td>
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</tbody>
</table>

and still is used extensively for the polyhydric alcohols. As early as 1962, House, et al. (39) reported the separation of sorbitol and mannitol hexaacetates on 1% QF-1. This principle was used by Jones, et al. (40) to determine sugar alcohols in dietetic biscuits. The acetate derivatives of 1,4-sorbitan, xylitol, D-mannitol, and D-sorbitol were separated and measured by GLC on a stationary phase consisting of 7% QF-1 and 1.7% BDS. The elution order was in the order stated with 1,4-
sorbitan as the internal standard. Later, Hundley (41) described the GLC determination of sorbitol in various bakery products, wines, and vinegars as its hexaacetate derivative. Mannitol and sorbitol hexaacetates were not separated on the column of choice, 10% DC-200. Bertrand and Pissard (42) described a procedure for the GLC determination of sorbitol in wines as its acetate. An ion-exchange clean-up to remove sugars and other interferences was employed followed by chromatographic determination on 7% QF-1.

GLC procedures for the determination of micro amounts of cyclamates have been described using trifluoroacetylation (43) and heptafluorobutyrylation (44) with subsequent GLC/EC determination. In the former (43) sodium cyclamate was quantitatively converted to the N-trifluoroacetyl derivative of cyclohexylamine by treatment with trifluoroacetic anhydride at 90°C for 1 hour, whereas in the latter (44), triethylammonium cyclamate was converted to the corresponding heptafluorobutyryl (HFB) derivative with heptafluorobutyric anhydride at 90°C for 1 hour.

The separation of several polyhydric alcohols as their 4-nitrobenzoates by HPLC has been recently reported (45). Arabitol, xylitol, mannitol, sorbitol, and maltitol were separated on a LiChrosorb Si 60 column in the order given.

Trimethylsilylation. In addition to acylation, trimethylsilylation has been used extensively with the polyhydric alcohols. Jones, et al. (40) in their studies on the determination of polyhydric alcohols in dietetic biscuits, used GLC of the TMS derivatives on 10% SE-30 to estimate the total sorbitol/mannitol content. The elution order was xylitol, 1,4-sorbitan (internal standard), and total hexitols. Fernandez-Flores and Blomquist (46) also reported on the determination of sorbitol in raisins and dietetic foods by GLC of its TMS derivative on SE-30, and Moseley, et al. (47) reported on its determination in cooked sausage products. Again sorbitol and mannitol could not be separated on these phases.

Saccharin has also been determined in foods and feeds as its TMS derivative (48), and glycyrrhizin as the TMS derivative of its aglycone (49).

Degradation Techniques. Probably the most convenient determination of cyclamates in soft drinks was that first described by Rees (50) who determined the cyclohexene produced after reaction of the cyclamates with nitrous acid by GLC. This technique was subsequently slightly modified by Richardson and Luton (51) to avoid possible losses of cyclamate and also studied in more detail by Dalziel, et al. (52). The last authors investigated the use of stronger nitrosating conditions which led to increased production of the nitrocyclohexene which they measured. However, they concluded that determination via the cyclohexene was more precise and accurate. Groebel and Wessels (53), using headspace analysis, also determined the cyclohexene produced.

Derse and Daun (54) determined cyclamates in urine and feces by GLC of the cyclohexylamine produced after hydrolysis of the parent compound with mineral acid and peroxide. Johnson, et al. (55), however, have reported that acid-peroxide hydrolysis does not produce a quantitative yield of cyclohexylamine and that a 7 hour period with HCl at 125°C and 15 psi in an autoclave is necessary for quantitative yield. Possibly a combination of this cleavage (55) with the GLC determination of Howard, et al. (56) would yield better methodology. The cyclohexylamine produced can also be derivatized with picryl chloride (57) or 1-fluoro-2,4-dinitrobenzene (58) to yield derivatives sensitive to EC detection and thus yield more sensitive methodology.

Saccharin has been determined in foods after Zn/HCl reduction to the thiophenol (59) and subsequent GLC analysis. Aspartame and several of its degradation products, including the diketopiperazine, have been determined after silylation with N,O-bis(trimethylsilyl)acetamide (BSA) (60).

Antioxidants

Antioxidants are generally used to prevent oxidative degradation and thus improve the flavour stability of fats, oils, or high fat foods. Most of the important antioxidants are listed in Table III. For regulatory purposes, it is often necessary to quantitate these antioxidants in various fatty foods where several antioxidants may be permitted and used either singly or in combination at levels up to 200 ppm.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Description</th>
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<tbody>
<tr>
<td>BHA</td>
<td>Butylated hydroxyanisole</td>
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<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
<tr>
<td>TBHQ</td>
<td>Mono tert-butylhydroquinone</td>
</tr>
<tr>
<td>Ionox-100</td>
<td>4-Hydroxymethyl-2,6-di-tert-butylphenol</td>
</tr>
<tr>
<td>THBP</td>
<td>2,3,5-Trihydroxybutyrophenone</td>
</tr>
<tr>
<td>PG</td>
<td>Propyl gallate</td>
</tr>
<tr>
<td>OG</td>
<td>Octyl gallate</td>
</tr>
<tr>
<td>DG</td>
<td>Dodecyl gallate</td>
</tr>
<tr>
<td>NDGA</td>
<td>Nordihydroguaiaretic acid</td>
</tr>
<tr>
<td>DLTDP</td>
<td>Dilauryl 3,3'-dithiodipropionate</td>
</tr>
<tr>
<td>TDPA</td>
<td>3,3'-Thiodipropionic acid</td>
</tr>
</tbody>
</table>

GLC methods have been widely used for antioxidant analysis. BHA, BHT, TBHQ, and Ionox-100 have been successfully quantitated without derivatization, whereas NDGA, THBP, TDPA, PG, OG, and DG, the polar, nonvolatile antioxidants, must be derivatized prior to GLC. Methods for the isolation and derivatization of these polar antioxidants may also include BHA, BHT, TBHQ, and Ionox-100, which would then be determined concurrently. DLTDP is normally not derivatized.

Of the derivatization techniques normally applied to phenolic compounds, trimethylsilylation is by far the most widely used. Methylation and acylation have found limited use. It should be emphasized that as antioxidants are normally used in fats, oils, or fat-containing foods, it is important to isolate the antioxidant from the food matrix without coextracting interfering lipid material before derivatization.

Trimethylsilylation. The conversion of the phenolic hydroxyl group of the antioxidant to the stable, GLC volatile TMS ether forms the basis of many analytical procedures. Nishimoto and Uyeta (61) reported the analysis of the TMS derivatives of alkyl gallates, ethyl protocatechuate and guaiac resin in food extracts using three stationary phases. The analysis of PG and THBP, and the confirmation by mass spectrometry of BHA, PG, Ionox-100, and THBP as their TMS derivatives in lard sublimates has been carried out by McCaulley, et al. (62) and Fazio, et al. (63). The antioxidants were silylated at room temperature in 5 minutes by the method of Sweeney, et al. (64) using pyridine:hexamethyldisilazane (HMDS):trimethylchlorosilane (TMCS) (5:3:1).

Kato, et al. (65) studied the retention characteristics of BHA, BHT, NDGA, PG, several other gallate esters, and other compounds as their TMS derivatives on three silicone and two polyester stationary phases. The derivatives were prepared
using BSA. Wachs and Gassman (66) silylated BHA, and ethyl, propyl, butyl, octyl, and decyl gallate in a method reportedly suitable for the determination of these antioxidants in edible fats. Silylation was performed with neat BSA for 15 minutes at 40°C.

The analysis of 2- and 3-BHA, TBHQ, PG, and NDGA as their TMS derivatives in extracts of vegetable oil has been reported (67). Silylation was carried out with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMCS in pyridine. BHT can also be silylated by this procedure. Groebel and Wessels (68) silylated PG, OG, and DG using neat BSA and the derivatization was applied to extracts from various fatty foods. The derivatization of BHA, BHT, PG, OG, DG, NDGA, TBHQ, and ethyl gallate using BSTFA with 1% TMCS (50°C for 30 min) has been reported by Karleskind and Valmalle (69). It is unlikely that BHT is derivatized by this procedure, as work in the authors' laboratory as well as that by Stoddard (67) indicate that TMS-BHT would elute after TMS-TBHQ on a nonpolar column, whereas nonderivatized BHT elutes before. In addition, the more readily derivatized TBHQ was incompletely silylated giving two peaks, presumably the mono- and di-TMS derivatives. The derivatization procedure was applied to intact oil, lard, and margarine samples with the resulting mixture directly injected onto the gas chromatograph to determine BHA, BHT (probably not silylated), PG, and ethyl gallate at relatively high levels.

Kline, et al. (70) recently reported the silylation of TBHQ, TDPA, PG, THBP, and NDGA in a variety of food extracts, using a similar reagent to that of Sweeley, et al. (64). The derivatives were quantitated on a 3% OV-225 column.

Early silylations of antioxidants were accomplished using HMDS and TMCS in a suitable solvent. This reagent was shown to derivatize PG, THBP, BHA and Iinox-100 (62), as well as TDPA and NDGA (70). With the availability of more powerful silylating agents such as BSA, BSTFA, and N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), even the highly sterically hindered BHT, can be derivatized. Although prolonged heating with BSA is reportedly necessary to derivatize the 2,6-di-tert-butylphenolic type of compound (71), silylation using BSTFA or MSTFA in a suitable solvent is more readily carried out. Stoddard (67) reports the silylation of BHT in pyridine by heating 2 hours at 120°C using BSTFA with 1% TMCS. Studies in the authors' laboratory have shown that BHT in pyridine can be completely silylated in less than 10 minutes at 120°C using MSTFA. It was observed that the pyridine used must be stored over potassium hydroxide to obtain this result. Highly purified, dry pyridine not stored over potassium hydroxide was not suitable. A chromatogram of nine antioxidants silylated by this technique is shown in Figure 3.

**Methylation.** Methylation of the phenolic hydroxyl to give the aryl methyl ether has been occasionally used to facilitate the GLC analysis of antioxidants. McCaulley, et al. (62) and Fazio, et al. (63) used diazomethane in ether to methylate TDPA in their procedures for the identification of several antioxidants in lard. They reported that silylation of TDPA was ineffective. Kato, et al. (65) prepared methylated derivatives and reported the chromatographic characteristics of BHA, BHT, NDGA, PG, several other gallate esters, ethylproocatechuate, and α-tocopherol on five stationary phases. Methyl iodide and sodium hydride were used to prepare the methylated BHA, BHT, and α-tocopherol. Octyl gallate in beer was extracted and determined by capillary GLC as the trimethyl ether after reaction with diazomethane with methanol catalyst as described by Silbereisen and Wagner (72). PG, OG, and DG have been determined in various food extracts as their methyl ether derivatives after methylation with diazomethane (68,73).

**Acylation and Other Procedures.** Kato, et al. (65) have investigated the gas chromatographic characteristics of the trifluoroacetates (TFA) and acetates of BHA, NDGA, PG, several other gallate esters, ethyl protocatechuate, and α-tocopherol. Some of the gallate trifluoroacetates were found to be unstable at high temperatures on polar columns. Dilli and Robards (74) prepared the TFA derivative of BHA and determined its detection limit (0.5 ng 3x noise level) using the ECD. Page and Kennedy (75) have described the direct determination of 2- and 3-BHA, TBHQ, and PG as their HFB derivatives in oils using EC/GLC. The detection limit of the BHA derivative, prepared as described by Ehrsson, et al. (76) was approximately two orders of magnitude less than that of the TFA derivative prepared by Dilli and Robards (74).

Ethoxyquin has been determined by GLC in apples as the ethoxyquin-HFB derivative using EC detection (77). The lower limit of detection of the HFB derivative was 0.02 ng, allowing detection at 0.005 ppm and quantitation of 0.05 ppm. A method for the determination of DL TDP based on the GLC analysis of lauryl alcohol released on hydrolysis has been described by Sedlar, et al. (78).

**Preservatives.**

Benzoi acid, sorbic acid, propionic acid, and the methyl, ethyl, and propyl esters of p-hydroxybenzoic acid represent the most commonly used preservative in the food industry. In the analysis of these preservatives, aside from the derivatization, problems in the isolation or extraction are often encountered. Generally, two extraction procedures may be employed. Steam distillation followed by extraction of the distillate, concentration, and derivatization suffers from incomplete recoveries of the p-hydroxybenzoates (parabens) in the distillation step (79,80). The other procedure, in which the acidified food sample is directly extracted with an organic solvent, may give incomplete recoveries with some foods as the solvent may not effectively penetrate the sample (79). In both these procedures, volatile preservatives or their derivatives may be lost...
during concentration or handling of extracts. A Kuderna-
Danish evaporator or similar apparatus may be used to pre-
vent losses on concentration (79,81). The parabens may be
partially hydrolyzed by base (79,82,83), which prohibits the
use of basic extraction procedures, however, a column cleanup
step can be used (83,84).

Although most preservatives can be determined by direct
GLC without derivatization, derivatization has been shown to be
advantageous in most instances. Silylation and methylation
procedures have been used extensively to convert the phenolic
hydroxyl or polar carboxylic acid group to derivatives suitable
for GLC. Other derivatives are not widely used.

\textit{Alklyation}. Diazomethane has been widely used to methyl-
ate final food extracts prior to the GLC determination of pre-
servatives. Early work by Goddijn and co-workers (81) using
diazomethane in ether-methanol, demonstrated the ready
methylation of the carboxylic acid group and the prolonged (1
hr) reaction time necessary for the methylation of the \( p \)-hy-
droxyl group of \( p \)-hydroxybenzoic acid. They determined ben-
zoi acid, several halobenzoic acids and several parabens in
fruit juices, syrups, and preserves using two GLC columns.
Groebel (85) described a similar methylation for the determin-
ation of sorbic acid, benzoic acid, and the parabens in various
fatty foods. Sorbic and benzoic acid have also been deter-
mined in wine after acidification, extraction, and methylation
with diazomethane (86). Silberesien and Wagner (72) demon-
strated the separation of 22 antioxidants and preservatives
after treatment with diazomethane, using both capillary and
conventionally packed columns. More recently, Iwaida and
co-workers (87) described a procedure for the determination
of sorbic acid in dried figs using diazomethane. The methylat-
ing agent, diazomethane, has been reported to react with the
double bond of sorbic acid to give undesirable side reactions
(83).

Other than diazomethane, esters of the preservative acids
can also be prepared by the action of acid and alcohol. Meth-
ylation with BF\(_3\)-methanol has been used to determine sorbic
acid in wine (88) and to confirm the presence of sorbic, ben-
zoi, salicylic, anisic, and cinnamic acid in other foods (82).
Benzoic, salicylic, 4-chlorobenzoic, and 4-hydroxybenzoic
acids have been determined in wine after esterification with
HCl-methanol (89). Ethylation, using \( \text{H}_2\text{SO}_4\)-ethanol, to de-
termine sorbic acid in fruit preparations (90) and wine (91) has
also been employed. Propionic acid has been determined as its
\( p \)-chlorophenacyl ester (92). This derivative can be determined
using either flame ionization or EC detection.

\textit{Silylation and Other Procedures}. Silylation techniques for
the preservative acids and the parabens are generally less time
consuming than methylation, as the paraben phenolic hy-
droxyl group is readily silylated. Vermeer and Dean (93) sily-
lated and separated the four possible sorbic acid isomers using
HMDS and TCMS in pyridine. The silyl derivatives of the
methyl, ethyl, propyl, and butyl parabens have been deter-
mied in soy sauce by Takemura (94). Sorbic acid, benzoic
acid, and the parabens were determined by Gosselé (79) as
their TMS derivatives in widely differing food products. The
procedure employed acidification, extraction, evaporation in a
Kuderna-Danish evaporator, silylation, and GLC. Of four
silylating methods examined, that with BSA provided the best
results. Yamashita, et al. (95) determined benzoic acid and
ethyl, propyl, and butyl parabens in soy sauce as their TMS
derivatives. Methyl, ethyl, and propyl parabens were extracted
from acidified samples, passed through a silica column, and
silylated with BSA in a procedure described by Daenens and
Laravelle (84). Larsson and Fuchs (83) employed separate isola-
tion techniques for benzoic and sorbic acid and for the para-
bens. The former were extracted into base and the latter puri-
fied on a silica column. Both final extracts were silylated using
MSTFA. Banda (96) also used two extraction procedures:
steam distillation for sorbic, benzoic, and salicylic acid, and
chloroform-ether extraction for \( p \)-hydroxybenzoic acid. The
TMS derivatives were prepared by the method of Sweeley (64).
In addition to alklyation and silylation, the ethyl, propyl,
isopropyl, butyl, and isobutyl parabens have been trifluoro-
acetylated and determined by FID (80). This procedure has
been applied to soy sauce distillates.

\textbf{Gums}

Gums may be defined as water dispersible or water soluble
polymeric thickening or gelling agents. These hydrophilic
 colloids or hydrocolloids are, simply stated, carbohydrates of
high molecular weight composed of one or more sugar moie-
ties linked in a specific manner. Gums are obtained from plant
seeds, tree exudates, seaweeds, and microbial sources.

Gas chromatographic analysis of gums normally involves
 cleavage of the polysaccharide into component sugars, deriva-
tization, and GLC. These procedures have been applied only
to the identification and characterization of various gums, and
not as yet to the analysis of gums isolated from foodstuffs.

Schmolck and Mergenthaler (97) describe the methanolysis
of a number of polysaccharide gums followed by silylation of
the liberated methyl glycosides. Guar gum, locust bean gum,
agar, carrageenan, algin, pectin, gum arabic, gum tragacanth,
gum karaya, and methyl cellulose were analyzed. Guar and
locust bean gum could be distinguished by their different
galactose to mannose ratios. The relative retention times and
peak areas of a number of individual silylated methyl glyco-
sides are given. The different anomeric forms of these deriva-
tives can give up to five peaks for one sugar, and for even
simple sugar mixtures evaluation of the chromatograms is
difficult (97,98). Artaud and co-workers (98) reported similar
problems for the silyl derivatives of galactose and mannose
from guar hydrolysates. However, they showed that the
reduction of the aldose to the alditol with sodium borohydride
followed by silylation resulted in a single peak for each sugar
and simplified the chromatogram. These workers (99) later
demonstrated that the acetates of the reduced gum hydroly-
zates exhibited more stability and were preferable to the TMS
er derivatives.

Varma, et al. (100) reported that acetylated aldonitriles,
which are stable derivatives of reducing sugars, gave single,
fairly well separated peaks. Guar gum and gum arabic were
hydrolyzed and the freeze-dried, neutralized hydrolyzate dis-
solved in pyridine and treated with hydroxylamine hydro-
chloride. The resulting sugar oximes, without isolation, were
then simultaneously acetylated and dehydrated to the aldo-
nitrile by further heating with acetic anhydride in the pyridine
(101). These derivatives have also been prepared by Varma, et
al. (101) after methanalysis-hydrolysis and from the neutral-
ized trifluoroacetic acid hydrolyzates of guar gum, locust bean
gum, agar, tragacanth, and methyl cellulose (102). Alduronic
acid components of polysaccharides are not determined by the
method described above (100-102). In order to identify
alduronic acid in polysaccharides, the acid-containing poly-
saccharide is first cleaved by methanolysis. The O-methyl-
alduronic acid methyl ester is reduced with sodium borohy-
dride to the methyl glycoside which is hydrolyzed to the
aldose. The aldose is determined as the acetylated aldonitrile
(103).
Methylation analysis as described by Merganthaler and Scherz (104) allows polysaccharide linkages of gums with identical sugar units to be identified. The polysaccharide is permethylated with sodium hydride and methyl iodide in anhydrous dimethyl sulfoxide. The methylated products are acid hydrolyzed and the resulting methylated sugars are determined by thin-layer chromatography or transformed to the aldonitrile acetates for GLC analysis.

Waxes

Natural waxes may be obtained in sufficient quantities for commercial purposes from a broad range of natural sources. These include the plant waxes, carnauba, candelilla, ouricouri, and Japan, the insect waxes, beeswax, Chinese insect, and shellac, and the marine waxes such as spermaceti wax. In addition, plant fossil waxes such as montan wax and especially paraffin wax are also important commercially. The totally hydrocarbon paraffin waxes, either synthetic or derived from petroleum, are not derivatized and will not be discussed in this report.

Beeswax, carnauba, candelilla, spermaceti, and shellac waxes are permitted for food use in Canada. Other countries permit additional or other natural waxes. Analytically, very little work has been directed toward the identification of waxes added to or used in foods. The major thrust of analytical methodology has been towards the characterization and quantitation of components of certain waxes, where derivatization plays an important role (105,106).

Gas chromatographic analysis of waxes may require liquid phases stable up to 400°C, such as SE-30, OV-1, or the carborane siloxane, Dexitil, especially when waxes are analyzed without pretreatment (105-108). Volatile hydrocarbons, alcohols, esters, and glycerides may be identified in the intact wax (105-108), even though over 50% of the wax components may not emerge from the column (105,106,108). A comparison of the chromatogram of the untreated wax with that after methylation with diazomethane may permit identification of the free fatty acids. Similarly, free alcohols, diols, and hydroxyesters may be converted to acetates or trimethylsilyl ethers; aldehydes may be converted to 0-methoxymethylene or reduced to alcohols with sodium borohydride (105,106).

A preliminary separation of wax components using conventional techniques such as column or thin layer chromatography is often employed to simplify wax analysis. The separated components may then be characterized and quantitated. Wax esters, either in the intact wax or as a separate fraction, can be cleaved with methanol-acid to give methyl esters and free alcohols (109-112,113,114,115-117) or saponified to give the free acid or salt and alcohol (118). The alcohols may be silylated (116,117), acetylated (111,112,115,117) or trifluoroacetylated (109) and determined by GLC or separated from the other wax components by thin-layer or column chromatography prior to derivatization. After saponification the free acids or salts are usually converted to methyl esters before GLC (113,114,115,117).

Equivalent chain-lengths of methyl trans-2-alkenoates, acetates of methyl ω-hydroxy alkanoates, primary and secondary alkanols and their acetates, alkanes, long-chain esters of long-chain alcohols, and several other wax components relative to methyl alkanoates have been compiled for Dexitil 300, SE-30, and other liquid phases and facilitate identification of these components (105,106,110). The use of appropriate synthetic compounds as internal standards aids provisional identification and quantitation (105,106,108).