Acid I was weak and did not show up very well on paper chromatograms. It was easier to see when rechromatographed on paper after being separated from most of the other acids on a silicic acid column.

Acid J is probably citramalic, which has been reported in apple peel (*11*).

Table II. Quantitative Analysis of Fresh and Darkened Concentrate

	Meq. in 100 Ml. of 18.6° Brix Juice	
Acid	Fresh juice	Darkened concentrate (diluted)
Total acids	13.4	13.6
D	0.16	0.16
E	0.69	0.74
G	11.4	11.6
I, J	0.15	0.15
M, N	0.27	0.26

Acid K was detected on paper chromatograms only after recovery from a silicic acid column.

Acid L, like H, shows up only under the most favorable conditions. As chlorogenic acid is made up of quinic acid and caffeic acid, it is reasonable to suppose that a trace of caffeic acid could be present.

Acids M and N appear to be succinic and lactic, respectively. Both have been found in apple fruit (*3, 9*). They were adequately resolved only in the ethyl alcohol–ammonia solvent and there seemed to be more of N than of M.

Acids O and P are weak and might be glutaric and adipic, respectively.

Acid Q is also weak and forms a rather diffuse spot.

All these identifications are tentative and will have to be confirmed by actual isolation of the acid and formation of derivatives. All acids present in the fresh juice remained in the darkened concentrate and no diminution in their concentration could be detected, but a larger amount of each acid will have to be available in order to get a true quantitative picture. This can probably be done by running several samples through a large silicic acid column, combining all fractions except malic, and rechromatographing on a column of the usual size. These studies will be the subject of future research.

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Received for review May 13, 1955. Accepted September 15, 1955. The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

FOOD ANALYSIS

Determination of Benzoic and Salicylic Acids in Food Products

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Benzoic and salicylic acids have long been used for preservation of food. As benzoic acid in quantities not exceeding 0.1% is permitted under the Federal Food, Drug, and Cosmetic Act, it has been employed widely. Both the processor and regulatory authority therefore must make certain that the approved quantity has not been exceeded. Salicylic acid cannot legally be added, but as it is ordinarily more effective than benzoic acid, it occasionally finds its way into foods.

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The acidity of the medium in which these acids are used is of major influence upon their preservative action (*20, 22*). Among the products in which one or both of the acids may be expected are ketchup, fruit concentrates, jams, preserves, and margarines. In general, methods for the determination of the two acids are similar, in that they are commonly extracted from an aqueous solution acidified with hydrochloric acid with the use of some volatile organic solvent, which later may be evaporated off. Among the solvents used are ether (*12, 13*), chloroform (*1, 15, 16*), and benzene (*4*). Sometimes separation is effected by steam distillation (*24*). Final estimation has been made gravimetrically (*9*), volumetrically by titration of the acids in alcohol solution with a standard base, and in the case of the salicylic acid by formation of a colored compound with ferric ion (*18, 23*).

Methods for analyzing mixtures of benzoic and salicylic acids have received limited attention. A tentative method of the Association of Official Agriculture Chemists (*3*) for their estimation in a compound ointment involves extraction of the acids and determination of total acidity by titration with a standard base. The salicylic acid is determined bromometrically and the benzoic acid is calculated by difference from the total acidity.

A review of the literature indicates

Ultraviolet spectrophotometric techniques make possible improved procedures for the determination of benzoic and salicylic acids in food. Benzoic and salicylic acids have absorption peaks in ether solution at 227 and 236 mμ, respectively. Salicylic acid has a secondary maximum near 306 mμ. The sodium salts in aqueous solution show similar absorbancies, but at slightly higher wave lengths and of different intensity values. Examination at selected wave lengths makes possible quantitative determinations of either acid alone or both in mixtures of the two. Benzoic acid has been determined spectrophotometrically in samples of ketchup and orange-base concentrate after a preliminary extraction with ether. The benzoate may be extracted directly from margarine samples with dilute sodium hydroxide. The extract is clarified with alumina cream before spectrophotometric examination. The methods are simple, easily carried out, applicable to low concentrations, and free of errors characteristic of other methods. They offer promise of use for a number of acids of similar type.

that benzoic acid and its salts absorb strongly in the ultraviolet region. Kumler and Strait (*14*) observed that in 0.01*N* sodium hydroxide there is an absorption peak near 224 mμ with a log ε value of 3.92, while the acid in water has a peak near 225 mμ with a log ε value of 3.96. Friedel and Orchin (*7*) report a λ_{max} at 230 mμ and a log ε of 4.1 for benzoic acid in cyclohexane. With salicylic acid, Piaw (*19*) states that peaks are observed at 235 and 300.6 mμ for the aqueous solution of the acid, and near 228 and 295 mμ for the sodium salt; a slight shift toward a longer wave length is indicated as the concentration is increased.

Previously Halban and Eisenbrand (*10*) had made a Beer's law study of the absorption of solutions of salicylic acid in alcohol at the secondary maximum near 303 mμ, and at the adjacent minimum near 254 mμ. These observations demonstrated that the intensity of the absorption of each acid in the ultraviolet region is of sufficient magnitude to serve as basis for a sensitive method for the determination of either acid. Furthermore, the fact that salicylic acid has a peak near 300 mμ while benzoic acid shows no absorption in this region suggests that this property will be of value in the analysis of a mixture of the two acids. However, because the absorption is dependent upon the solvent and the nature of the species in the solution, a quantitative method requires careful examination and control of the conditions of the determination. Preliminary experiments in this laboratory (*17*) having shown promise of success, the work has been continued and the method developed has been adapted to the examination of several food products.

Experimental

A Cary Model 11 recording spectrophotometer was employed where continuous absorption curves were to be established. The Beckman Model DU instrument was used in many instances when absorbancies at a limited number of wave lengths were to be determined. Curves showing conformance to Beer's law were prepared with both instruments.

As it was planned to extract the benzoic and salicylic acids from aqueous solution into ether, absorbancies of the two acids were evaluated first in this solvent. For experiments where the aqueous solutions could be employed, the absorbancies were evaluated for the sodium salts of the acids. Representative absorption curves are shown in Figure 1. These are of the same nature as those reported by other investigators, but minor difference in intensities and position of maxima result from differences in solvents and conditions of examination.

In the ether solutions of the acids, the absorption peaks are slightly displaced toward the side of longer wave length and the intensity of the absorption is increased markedly. Table I shows that the intensity of the peak at the maximum of shorter wave length increases slightly with decrease in pH in the alkaline range, but that a very large increase takes place when the pH is brought into the acidic range. However, the maximum for the salicylate at the longer wave length is practically constant over a considerable change in pH. It is apparent that any quantitative study must involve careful consideration of the solvent and the pH of the solutions.

Another factor of significance is the nature of the basic solution. Sodium hydroxide (1*M*) starts to absorb at 230 mμ; sodium carbonate, at about 240 mμ. Although their maxima lie further out in the short ultraviolet range, the side bands for high concentrations of these compounds overlap the region of 224 to 231 mμ in which benzoic and salicylic acids exhibit maxima. Consequently it is necessary to have a reference solution containing the same quantities of these reagents, so that their effects may be compensated when a solution containing these reagents is examined.

Tables II and III show absorbancy data for several sets of conditions in which the concentrations of the salts in water and the acids in ether were varied. These absorbancy data, when plotted, indicate a linear relationship to concentration and satisfactory conformance to Beer's law.

Table I. Absorbancies of 0.0001*M* Benzoate and Salicylate under Different Conditions of Solution

Solution	Benzoate		Salicylate			
	λ_{max}, mμ	*A*	λ_{max}, mμ	*A*	λ_{max}, mμ	*A*
Sodium salts in water						
At pH 13.0	224	0.820	231	0.680	297	0.340
At pH 8.8	224	0.852	231	0.673	296	0.340
At pH 8.0	224	0.866	231	0.673	296	0.340
At pH 3.62	229	1.114	234	0.729	298	0.340
Acids in ether	227	1.230	236	0.780	306	0.402

Absorbancies in 1-cm. cells.

Table II. Absorbancies of Benzoic and Salicylic Acids in Ether Solution

Concentration		Absorbancy in 1-Cm. Cell		
Moles × 10^5	Mg./100 ml.	λ 227 mμ	λ 236 mμ	λ 306 mμ
		Benzoic Acid		
4.5	0.65	0.551	...	...
9.0	1.30	1.167	...	...
13.5	1.95	1.684	...	...
18.0	2.60	2.279	...	...
22.5	3.25	2.875	...	...
		Salicylic Acid		
5.0	0.80	0.227	0.356	0.207
10.0	1.60	0.530	0.780	0.402
15.0	2.40	0.819	1.194	0.624
20.0	3.20	1.127	1.625	0.869
25.0	4.00	1.356	1.950	1.057

Table III. Absorbancies of Sodium Salts of Benzoic and Salicylic Acid in Aqueous Solution

Concentration		Absorbancy in 1-Cm. Cell	
Moles × 10^5	Mg./100 ml.	λ 224 mμ	λ 296 mμ
Sodium Benzoate			
6.34	0.915	0.53	...
8.74	1.260	0.73	...
12.20	1.750	1.00	...
13.93	2.010	1.15	...
Sodium Salicylate			
2.60	0.416	0.175	0.084
6.52	1.041	0.436	0.225
13.00	2.082	0.860	0.445
16.30	2.602	1.100	0.570

Although the salicylic acid maximum occurs at 236 mμ, for purposes of analysis of mixtures of the two acids in ether solutions, the maximum for benzoic acid at 227 mμ was selected for observation of the combined absorbancies. Hence values are reported for salicylic at 227 mμ as well as the maximum at 236 mμ.

Analysis of Mixtures of Benzoic and Salicylic Acids

Of considerable interest in the present study was the possibility of determining benzoic and salicylic acids when present together. Gravimetric and volumetric methods are particularly difficult when the quantities are small. It has been pointed out (Figure 1) that 0.0002*M* salicylate shows marked absorption near 300 mμ (306 in ether and 297 mμ in water) where benzoic acid has no absorption. Hence, from an evaluation of absorbancy in this region a direct spectrophotometric determination of salicylate is possible. Then, from the amount of salicylate indicated the corresponding absorption at 224 mμ in water (or 227 mμ in ether) may be calculated. By subtraction of this value due to the salicylate from the observed total value at this lower wave length the residual absorption due to benzoic acid can be obtained. Finally, from known absorbancy-concentration relationships for the benzoic compound, its quantity may be estimated.

The data in Tables II and III for absorbancies of the acids in ether and the salts in water at the desired wave lengths may be plotted to secure the necessary working curves for calculating the amounts of each compound for a given set of conditions. Table IV gives the results of a series of analyses of ether solutions in which the proportions of the acids in the mixture were varied. The quantity of salicylic acid is found directly from the absorbancy at 306 mμ. Then the absorbancy at 227 mμ (column *b*) is noted from the appropriate working curve from Table II. Column *a* − *b* in Table IV gives the residual absorbancy at 227 mμ due to benzoic acid, from which its quantity may be found by reference to a working curve for this acid at 227 mμ.

Table V gives the final values for a similar series of analyses of mixtures of

Figure 1. Absorption curves for benzoic and salicylic acids and their sodium salts examined in 1-cm. cells

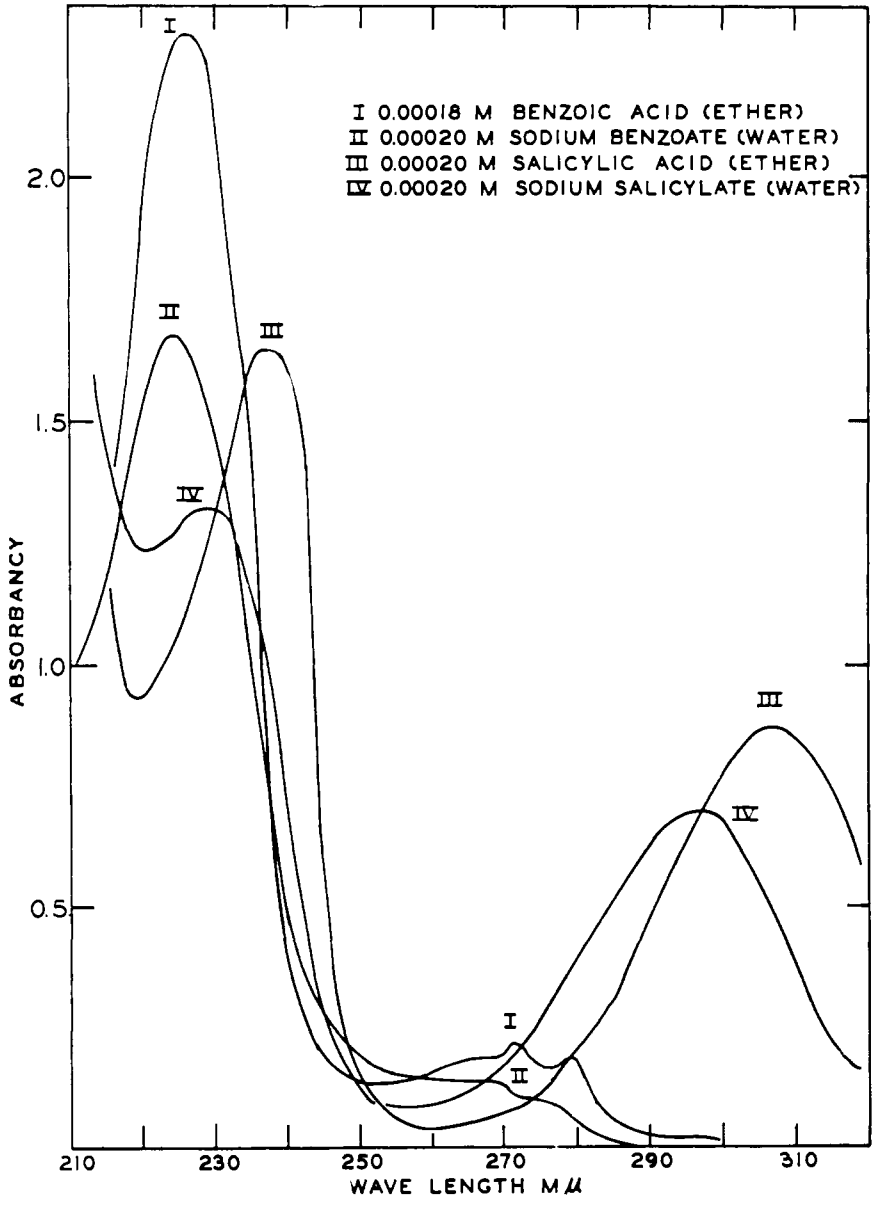

Table IV. Analysis of Mixtures of Salicyclic and Benzoic Acids in Ether Solution

Sample	Salicylic Acid: Absorbancy at 306 mμ	Salicylic Acid: Moles × 10⁵ Taken	Found	Diff.	Absorbancy at λ 227 Mμ: a Total	b Salicylic	a − b Benzoic	Benzoic Acid, Moles × 10⁵ Taken	Found	Diff.
1	0.208	5.00	4.90	−0.10	0.823	0.268	0.555	4.50	4.50	0.00
2	0.098	2.50	2.25	−0.25	1.000	0.122	0.878	6.75	7.00	+0.25
3	0.313	7.50	7.40	−0.10	0.688	0.405	0.283	2.25	2.35	+0.10
1	0.220	5.00	5.10	+0.10	0.856	0.280	0.576	4.50	4.65	+0.15
2	0.112	2.50	2.60	+0.10	1.035	0.143	0.892	6.75	7.15	+0.40
3	0.321	7.50	7.50	0.00	0.682	0.410	0.272	2.25	2.25	0.00

the sodium salts in aqueous solutions, based on the working curves from Table III. For the calculation of percentages of the preservatives in food products parts per million or some similar weight per unit volume is more convenient than moles for the expression of concentration. These units are used in Tables V and IX.

The difference between added and experimentally indicated quantities is considerable in several instances. These relationships could undoubtedly be improved by more refined conditions of experimentation—for example, special equipment for minimizing of evaporation of solvent where ether solutions are employed would be advantageous. Because the absorbancy of the salicylate at the longer wave length is lower, longer cells could be employed at this step to bring the absorbancy into the optimum range for the examination. Because any error in this value will contribute to the subsequent calculation of the absorption of the salicylic acid at the shorter wave length and the residual benzoic acid absorbancy, the measurement is of particular significance. This is shown in sample 2a of Table V. The data given in Tables IV and V illustrate the principles of the method and results to be obtained under ordinary conditions.

Ultraviolet Absorption in Determination of Benzoate in Food

After the necessary preliminary information had been secured relative to the absorption characteristics of the two acids, the procedure was tested upon representative food products.

As a container of ketchup is seldom emptied soon after opening, it is a type of product for which additional preservative action may be desired and one to which benzoate commonly has been added. Therefore, ketchup was selected as one of the materials to be examined.

Determination of Benzoate in Ketchup Samples, whose labels indicated that no benzoate had been added, were tested qualitatively and the absence of the preservative was confirmed. To these samples were added known amounts of sodium benzoate, and the mixtures were subjected to analysis. Because of the pulpy nature of the material, it is difficult to remove the solution containing the benzoate. Preliminary experimentation led to a modification of the official procedure.

Procedure A sample of 50 grams of ketchup is weighed into a beaker, 10 grams of fine salt is added, and the solution is made basic with 10% sodium hydroxide. To this mixture is added about 2 grams of filter aid material (Johns-Manville Super-Cel). The mixture is then transferred to a 1-liter graduated flask with saturated salt solution, make up to volume with the salt solution, thoroughly mixed, and allowed to stand for an hour.

A piece of canvas filter cloth is cut so as just to fit into a 15-cm. Büchner funnel. This is connected to a suction flask and the cloth is covered with a thin slurry of the filter aid from a suspension in water. The water is removed by suction, and the mat is washed gently with distilled water and dried by drawing air through. The original suction flask is replaced with a clean dry one and the ketchup mixture is filtered with the aid of suction. The entire operation requires only about 20 minutes. A 100-ml. portion of the filtrate is placed in a 250-ml. separatory funnel, and neutralized with 3 to 1 hydrochloric acid and an excess of 5 ml. of acid is added. The solution is then extracted with four portions of ether in successive volumes of 35, 25, 20, and 10 ml.

During the extraction the funnel is shaken vigorously and the two layers are allowed to separate for 5 minutes. The lower aqueous portion is drawn off to a stage such that about 10 ml. of the mixed aqueous solution remains. Then the mixture is again shaken vigorously and the lower water layer is drawn off as completely as possible. The residual ether layers for each portion are drawn off and combined in a 100-ml. volumetric flask. After the last extraction, the separatory funnel is washed with about 10 ml. of ether. Finally the flask is filled to the mark with ether and the contents are thoroughly mixed. From this solution, a 10-ml. aliquot is pipetted into a 100-ml. flask and diluted to volume with the solvent. The diluted solution is examined with the spectrophotometer at 227 mμ. The absorbancy noted is referred to the appropriate standard

Table V. Analysis of Mixtures of Sodium Salicylate and Sodium Benzoate in Aqueous Solutions

Sample	Absorbancy at 296 mμ, 1-Cm. Cell	Salicylate, P.P.M. Taken	Found	Diff.	Absorbancy at 224 mμ, 1-Cm. Cell, Total	Benzoate, P.P.M. Taken	Found	Diff.
1 a	0.118	5.20	5.30	+0.10	0.520	4.92	5.1	+0.18
b	0.118	5.20	5.30	+0.10	0.510	4.92	4.9	−0.02
2 a	0.163	6.51	7.30	+0.81	0.410	2.46	1.70	−0.76
b	0.146	6.51	6.50	−0.10	0.420	2.46	2.50	+0.04
3 a	0.176	7.81	7.90	+0.10	0.474	2.46	2.40	−0.06
b	0.174	7.81	7.80	−0.01	0.458	2.46	2.20	−0.26
4 a	0.061	2.60	2.65	+0.05	0.552	7.38	7.60	−0.22
b	0.060	2.60	2.60	0.0	0.534	7.38	7.3	−0.08
5	0.205	8.67	9.20	+0.55	0.486	1.64	1.65	+0.01

Table VI. Determination of Sodium Benzoate in Ketchup

(Estimation after extraction with ether from acid solution)

Sample	Absorbancy of Ether Solution; 1-Cm. Cell at 227 Mμ	Sodium Benzoate ⇌ Benzoic Acid at Stage of Absorbancy Measurement Mg./100 Ml.		% in Original Ketchup		
		Added	Recovered	Added	Found	Diff.
1	0.64	0.724	0.730	0.144	0.146	+0.002
2	0.49	0.559	0.560	0.112	0.112	0.0
3	0.38	0.459	0.445	0.092	0.089	−0.003
4	0.78	0.918	0.900	0.183	0.180	−0.003
5a[a]	0.72	1.150	0.830	0.230	0.160	−0.07
5b	0.98	1.150	1.130	0.230	0.226	−0.004

[a] Extracted by swirling instead of vigorous shaking.

working curve for the corresponding concentration of benzoate.

The results for the determinations are given in Table VI. As 50-gram samples are diluted to 1 liter and 100 ml. of this solution is extracted into 100 ml. of ether, 10 ml. of which are diluted to 100 ml. at the stage of final spectrophotometric examination, the milligrams per 100 ml. at this stage is 1/100 of original amount present. Therefore

$$\left(\frac{100 \times \text{mg.}}{50{,}000}\right) \times 100 = \% \text{ in original sample}$$

All solutions were shaken vigorously in the ether extraction except sample 5a, which was swirled gently during extraction. The low results obtained are in agreement with the observation of Randall (*21*), who recommended vigorous shaking as the preferred method of extraction.

Discussion of Extraction Procedure. The use of a 50-gram sample of Ketchup instead of the 250 grams employed in the official method, with dilution to 1 liter instead of 500 ml., is possible because of the much greater sensitivity of the method for finally measuring the benzoate. The greater dilution contributes to an improved flocculation of the tomato pulp and easier removal of the solution portion. The use of filter aid and the type of the filtration process indicated is highly advantageous over the slow and tedious operation of the official method, in which the more viscous material is filtered through a fluted filter paper medium, an operation that frequently requires several hours. Use of chloroform for extraction of the acids from aqueous solution is advantageous, in that it has a higher specific gravity and hence the extract may be more conveniently drawn off. However, chloroform favors formation of emulsions which are hard to break, and has such low transparency in the shorter ultraviolet region that measurements at 227 mμ are difficult. Ether was found much more satisfactory in these last two respects. The fact that hydrochloric acid and certain interfering organic acids are less soluble in chloroform than ether may have advantages when the benzoic or salicylic acid is to be estimated by a volumetric method, but it is of no significance when the ultraviolet absorption method is used, as their presence is ordinarily without influence upon ultraviolet absorption. Because the absorbancy of benzoic (or salicylic) acid is very great, much smaller initial samples may be used than are necessary for a titration method. As indicated in Table VI, the results are satisfactory.

One special precaution should be noted when ether is used. Many samples contain traces of impurities which show absorption in the ultraviolet region. Frequently, extractives from rubber gaskets and other materials with which the ether may come in contact in demountable spectrophotometer cells give rise to absorption. Consequently, ether of the best quality obtainable should be taken and solvent should be taken from the lot used as a reference solvent. Absorption cells with fused quartz windows are preferred over the demountable types.

Transfer of Benzoic Acid from Ether to Aqueous Solution Because of the volatility of ether and some other minor objectionable features characteristic of ether solutions, a study was made of the possibility of transferring the extracted benzoic acid from the ether back into aqueous solution before making the spectrophotometric examination. Accordingly, a 50-ml. portion of each ether extract obtained in the experiments of Table VI was placed in a separatory funnel and extracted successively with four portions (35, 25, 20, and 10 ml.) of 0.01% sodium hydroxide solution. The extracts were drawn off into a 100-ml. flask and made up to volume with the sodium hydroxide solution. The resulting solution for each sample was examined, using 0.01% sodium hydroxide as the reference solution (Table VII).

The recoveries are in agreement with the quantities originally added, even though slightly lower values were indicated for the ether solutions from which they were obtained. It is possible that in shaking the samples with the alkaline solution more carbon dioxide was absorbed by the sample than by the reference blank. Because sodium carbonate shows a slight general absorption in the region of 210 to 260 mμ, a slightly higher value might be attributed to this cause. However, the results may be occasioned by some experimental errors, characteristic of one or both of the extractions and measurements, as similar results were not observed in the later study of orange-base concentrates.

Determination of Benzoate in Orange-Base Concentrate The orange-base concentrate was obtained from a local bottling works. The statement that no benzoate had been added was confirmed by chemical examination.

A known amount of sodium benzoate was added to 25 grams of orange-base material and the remainder of the procedure was essentially the same as that used with ketchup samples.

Evaluations of the benzoate were made from the ether extracts of the acidified samples and from the sodium benzoate obtained from another series of samples by extraction of the ether solutions with sodium hydroxide (Table VIII).

The flocculations, filtrations, and extractions of the samples, performed as for the ketchup samples, gave no difficulty. A colorless extract was obtained which was free from turbidity and suitable for direct photometric examination. Even on long standing, the extracts appeared to be stable. The differences between added and recovered quantities were of the order of 1%.

Table VII. Determination of Sodium Benzoate in Ketchup

(Estimation after extraction with ether and then extraction from ether with aqueous 0.01% sodium hydroxide)

Sample No.	Absorbancy; 1-Cm. Cell at 224 Mμ	Sodium Benzoate, Mg./100 Ml. of Solution Examined	
		Added	Recovered in Aqueous Solution
3	0.27	0.459	0.46
4	0.53	0.918	0.92
5	0.66	1.150	1.15

Determination of Sodium Benzoate in Margarine As the products analyzed, ketchup and orange-base concentrate, were of similar nature, attention was directed to margarine material, which requires

different preliminary treatment. Extraction with ether is not practicable because of the solubility of the fat. In making an aqueous extract, the use of a strong alkali to bring the benzoate into an aqueous medium may lead to saponification of the glycerides. Friese (*8*) used a hot solution of sodium carbonate and followed with addition of barium chloride to the extract to precipitate the fatty acid. The filtrate was acidified and extracted with ether, and the residue from the ether was weighed.

Table VIII. Determination of Sodium Benzoate in Orange-Base Concentrate

Sample No.	Absorbancy in 1-Cm. Cell at 227 Mμ	Sodium Benzoate, Mg./100 Ml. Added	Sodium Benzoate, Mg./100 Ml. Recovered
From Extraction of Benzoic Acid into Ether Solution			
1	0.56	6.40	6.45
2	1.05	12.10	12.10
3	1.05	12.10	12.10
By Subsequent Extraction from Ether Extracts with 0.01% Sodium Hydroxide			
4	0.865	15.15	15.05
5	0.350	6.10	5.90
6	0.870	15.15	15.10

Hash (*11*) substituted ammonia for sodium carbonate, but in other respects his process is similar. Daels (*5*) used hot distilled water to separate the benzoate from the fat, then clarified the aqueous extract with potassium ferrocyanide and zinc sulfate to remove the protein material, and made the final extraction of the benzoic acid with ether. The method used by Davy (*6*) is similar to that of Friese, but instead of weighing the final residue he dissolved it in dilute alcohol and titrated it with standard sodium hydroxide. Grossfeld (*9*) experimented with a number of methods to remove the protein and later converted the benzoic acid to a product which could be estimated colorimetrically.

Samples of commercial margarine were obtained from a local source. According to the manufacturers, these contained a maximum of 0.1% sodium benzoate. A number of 10-gram samples were weighed into separatory funnels and extracted with four portions of warm 0.01% sodium hydroxide solution (1 ml. of 1% sodium hydroxide per 100 ml. of water). The extracts contained suspended matter and hence could not be examined directly in the spectrophotometer. Centrifugation of the solutions for 20 minutes at 1600 r.p.m. did not significantly improve their condition nor did passing the solution five times through a filter paper completely remove all suspended matter. The fat particles appeared to be removed, but a cloudiness, possibly due to colloidal protein, remained. A previously used clarification reagent (*3, 6*), involving an addition of zinc acetate followed by potassium ferrocyanide, was tried. A clear filtrate was secured, but further study indicated that the excess reagents adsorbed light appreciably near 230 mμ and below. An error due to this factor might have been compensated by use of a suitable blank, but it was decided to try other clarifying reagents.

An alumina cream reagent, prepared as specified in the official methods (*2*), was found to be satisfactory with respect to removal of suspended matter. Tests with solutions of known benzoate content showed no loss from the use of the reagent. As a result of this study, the following procedure was adopted.

Procedure. Weigh a 10-gram sample of the margarine into a separatory funnel. Extract successively with five 40-ml. portions of 0.01% sodium hydroxide solution. Draw off each portion as completely as possible into a 250-ml. graduated flask. Add to the solution about 5 ml. of alumina cream and bring the solution to a final volume of 250 ml. with the sodium hydroxide solution. Mix thoroughly, filter, remove a 25-ml. aliquot, and dilute to 100 ml. with distilled water. Examine the final solution for absorbance at 224 mμ and refer to the working curve for the quantity of benzoate.

Table IX. Determination of Sodium Benzoate in Margarine

Sample	Absorbancy in 1 Cm. Cell at 224 Mμ	Sodium Benzoate in Portion Examined, P.P.M.	Sodium Benzoate in Sample, %
1 a	0.51	8.80	0.088
b	0.52	9.00	0.090
c	0.54	9.40	0.094
d	0.53	9.20	0.092
2 a	0.635	11.00	0.110
b	0.660	11.45	0.114
c	0.660	11.45	0.114
d	0.628	10.90	0.109
e	0.660	11.45	0.114

Results of analyses employing the procedure are given in Table IX and are in satisfactory agreement in view of the small percentage present. One of the commercial margarines contained slightly less and one slightly more than the permitted maximum of 0.1% benzoate.

The properties of benzoate and salicylate are similar, and it has been shown by other workers that the salicylate may be extracted in the same way as benzoate. Hence, no experiments were conducted upon the addition and recovery of the salicylate from food materials. If present alone, it may be determined in the same way as benzoate at wave length 306 or 236 mμ. The presence of an absorption maximum near 300 mμ in an extract supposed to contain only benzoate would point to the presence of salicylate. If both acids are present, they may be determined by the method illustrated in Table IV.

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Received for review February 4, 1955. Accepted June 13, 1955.