Lemon Juice Composition. Identification of the Major Phenolic Compounds and Estimation by Paper Chromatography

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The general analytical procedure consisted of enzymatically hydrolyzing the phenolic glycosides in the juice, extracting the aglycones, chromatographing the extract and standards, spraying with chromogenic reagents, and estimating the amounts either by visual comparison or by densitometry. The average amounts of the phenolic aglycones measured in lemon juice after hydrolysis were (mg. of aglycone per 100 ml. of single-strength lemon juice): eriodictyol, 20; hesperetin, 1.4; quercetin, 2.2; phloroglucinol, 2.0; and umbelliferone, 0.2. The literature reports hesperidin as the major phenolic; a brief review describes the discrepancy. Hesperidin and diosmin were found in the cloud (insoluble, suspended material in juice).

ALTHOUGH the phenolics of lemon peel have been investigated quite extensively in the past few years by Horowitz and Gentili (*8–10, 12–14*) and others (*3, 26*), relatively little has been done with the juice. Bernhard (*1*) identified some of the coumarin compounds in the juice, 5-geranoxypsoralen, 8-geranoxypsoralen, 5-geranoxy-7-methoxycoumarin, 5,7-dimethoxy coumarin, oxpeucedanin hydrate, and byakangelicin.

Measurements of lemon juice phenolics have been limited by the lack of suitable methods. Davis (*4*) developed a nonspecific colorimetric procedure for measuring the total flavanones in citrus juices. He assumed that the major flavanone in lemon juice was hesperidin with an eriodictyol glycoside possibly present. Several limitations of the method were later pointed out (*11*). Rowell and Winter (*23*) developed a colorimetric method for the total flavanones in citrus bioflavonoids based on their borohydride reduction. Hörhammer and Wagner (*7*) developed a method for measuring total flavanones of citrus bioflavanoids and juices as their 2,4-dinitrophenylhydrazones. They reported the lemon juice flavanones as hesperidin.

Since the total phenolic fraction was useful in helping to characterize lemon juice (*21, 28*), a method by which the individual compounds could be analyzed was desired. Hagen and coworkers (*5*) recently developed a quantitative method for the individual flavanone glycosides of grapefruit. However, their method of column chromatography, thin-layer chromatography, and fluorescence analysis was not suitable for routine analytical work. Roux and Maihs (*22*) developed a general method for estimating individual flavonoid compounds by paper chromatography. Their approach, with some modifications, appeared to be the most practical for the analysis of the phenolic compounds in lemon juice. The purpose of the current work is to identify the major phenolic compounds (glycosides and aglycones) in lemon juice and to develop a suitable method for estimating quantitatively the composition of the lemon juice phenolic fraction.

Experimental

Hydrolysis and Extraction. Ten milliliters of lemon juice, previously clarified by filtration through Celite, was adjusted to pH 4.6 with concentrated NaOH. Hemicellulase (0.5 gram, Nutritional Biochemicals Corp.) was added and the mixture left overnight at 35° C. (*12*).

Approximately 4 volumes of acetone and 5 grams of Celite were added to the hydrolyzate. The mixture was filtered, and the residue was washed several times with acetone. The filtrate was concentrated to 3 to 4 ml. on a rotary vacuum evaporator. More acetone (about 30 ml.) was added, and the resulting precipitate was filtered through Celite. The filtrate was concentrated to about 1 to 2 ml. The residue was dissolved in a minimum of 95% ethanol and transferred to a 10-ml. volumetric flask.

General Procedure. The general procedure was essentially the same for all of the compounds measured, and the specific details for the individual compounds are listed in Table I. A sheet of Whatman No. 1 filter paper was spotted with the extract and standard, and developed in the proper solvent system. The developed chromatogram was dried, sprayed with the appropriate reagent, and the amounts were estimated visually by comparing spot densities with the standards. When the amount of the extract was bracketed closely by the amounts of the standard, the estimate was accurate within 20 to 50%.

A more precise estimate was made on a second chromatogram by spotting three replicates of the extract and standard so that each has approximately the same phenolic content. The standard and extract were developed and sprayed as above. When dry, the chromatogram was cut into strips and run through a Spinco Analytrol recording densitometer. Maximum sensitivity was achieved with the 1-mm. slit masked off to a 10-mm. height so as just to include the spot with little background. The areas under the resulting graphs were measured with a planimeter, and the phenolic content of the juice was calculated as follows:

$$C(\text{juice}) = \frac{\mu\text{g. (std.)} \times \text{area (ext.)} \times 100}{\text{area (std.)} \times \mu\text{l. (ext. spotted)}} \times (\text{mg.}/100 \text{ ml.})$$

In the original lemon juice, the phenolics were present as glycosides, but since they were measured as aglycones, they are reported here as mg. of aglycones per 100 ml. of single-strength lemon juice.

Spray Reagents. BIS-DIAZOTIZED BENZIDINE (*16*). The reagent consisted of two solutions, (a) benzidine hydrochloride (6 grams) dissolved in a dilute HCl acid solution (14 ml. of concd. HCl plus 980 ml. of H_2O) and (b) 10% $NaNO_2$ in water. Two parts of solution (b) were added to three parts of solution (a) and used immediately. The chromatograms were allowed to stand 2–3 minutes after spraying for complete color development. Then they were washed several times with water to remove the excess reagent.

AMMONIACAL SILVER NITRATE (*20*). The reagent was prepared just before use by adding 6*N* NH_4OH drop by drop to 14% $AgNO_3$ until the Ag_2O precipitate just dissolved. After the chromatograms had been sprayed, they were washed several times in distilled water, once in dilute $Na_2S_2O_3$ (0.15%), and again with water.

Table I. Experimental Details for Individual Compounds

Compound	Approximate Amounts[a] Extracts, μl.	Standard, μg.	Solvent System	R_f	Spray Reagents
Eriodictyol	10	1.0, 1.5, 2.0	Water–35% acetic acid[b]	0.55	Ammoniacal silver nitrate
Quercetin	10	0.2, 0.4, 0.6		0.14	
Hesperetin	20	0.3, 0.6, 0.9	Benzene–acetic acid–water (127:72:3)	0.95	Bis-diazotized benzidine
Phloroglucinol	10	0.2, 0.4, 0.6	Water	0.51	
Umbelliferone	20	0.01, 0.02, 0.04, 0.06	Water	0.55	UV fluoresence with NH_3[c]

[a] Approximate amounts of the sample extract and standards used for the visual estimation.
[b] Developed with distilled water to a height of 15 to 18 cm., dried, developed with 35% acetic acid in the same direction to a height of 12 to 15 cm.
[c] The level of umbelliferone was too small to react chemically with the spray reagent, so the amount was visually estimated by comparing the UV fluorescence of the extract and a series of standards.

Isolation and Identification of Eriodictyol. One liter of fresh lemon juice was hydrolyzed, and the phenolics were extracted as described above. The extract was concentrated and dried under vacuum onto 1 gram of Whatman No. 1 powdered cellulose. A 2.5 × 25 cm. column was prepared from an aqueous slurry of powdered cellulose and washed for 1 to 2 hours with water. A filter paper disk was placed on top of the column as well as by the powdered cellulose with the adsorbed extract. The column was developed with water, and the effluent was checked by UV spectra and paper chromatography. A fluorescent band of coumarins preceded the flavanones. The flavanone fraction consisted mainly of eriodictyol with some hesperetin and coumarins. After several recrystallizations from ethanol-water, a chromatographically homogeneous solid was obtained which melted at 263–9° C. and was chromatographically identical with authentic eriodictyol. The infrared spectra of the samples in KBr pellets agreed in every detail. The UV maxima and spectral shifts were also the same for both compounds: 289 mμ (EtOH); 327 mμ (with NaOAc); 310 mμ (with $AlCl_3$).

Isolation and Identification of Eriocitrin. To 400 grams of commercial lemon juice concentrate (400 grams of anhydrous citric acid per liter), two volumes of methanol were added. The mixture was stirred to dissolve the soluble components and coagulate the pectin. The pectin precipitate was filtered off and the filtrate adjusted to approximately pH 4.5 with concentrated NaOH. The partially neutralized citric acid was precipitated with 1 liter of acetone and filtered off. The filtrate was evaporated on a rotary vacuum evaporator to a sirup and dissolved in a minimum of methanol. Three volumes of ethyl acetate were added to the methanol sirup, and the procedure was repeated to remove the remaining citrates. The filtrate was evaporated to a sirup and dissolved in 100 ml. of methanol. To the methanol solution, an excess of a saturated solution of neutral lead acetate in 50% aqueous methanol was added. The lead precipitate of the *o*-dihydroxy flavonoids was centrifuged and washed several times with 50% aqueous methanol. (The supernatant and washings were saved for identification of phlorin.) The lead precipitate was suspended in methanol and saturated with H_2S. The PbS was removed by centrifuging. The residual precipitate was again suspended in methanol, saturated with H_2S, and centrifuged. The combined supernatant solutions were evaporated and chromatographed on a silicic acid column with methanol in $CHCl_3$ according to the scheme of Horowitz and Gentili (*12*).

The eriodictyol glycoside isolated from the juice was identical, in every respect, with authentic eriocitrin. The UV spectra and shifts for both compounds were: 284 mμ (ethanol) and 307 mμ (with $AlCl_3$). The nona-acetates of the compound from lemon juice and authentic eriocitrin [prepared by heating with acetic anhydride and sodium acetate (*12*)] had the same UV and IR spectra in KBr pellets. The R_f values in paper chromatography, thin-layer chromatography, and electrophoresis were also the same for both compounds.

Isolation and Identification of Phlorin. The phenolic fraction not precipitated by lead in the isolation of erioctrin contained phlorin. The excess lead was precipitated with H_2S and centrifuged. The supernatant solution was concentrated to a sirup, and a small amount of silicic acid was added. The mixture was taken to dryness under vacuum, powdered, and added to a silicic acid column in a $CHCl_3$ slurry. The column was developed with methanol-chloroform mixtures (from 5 to 25% methanol). Phlorin was eluted with 20% methanol. The main contaminant, eriocitrin, was removed by preparative thin-layer chromatography on powdered cellulose with water as the solvent. The material which was isolated from lemon juice cochromatographed with authentic phlorin (*10*) on both paper and thin-layer chromatography. The deep orange color produced with bis-diazotized benzidine was the same for both compounds.

A portion of the phlorin from lemon juice hydrolyzed with β-glucosidase (Mann Research Lab) in pH 4.5 acetate buffer yielded phloroglucinol and glucose. The phloroglucinol was identified by paper chromatography and the bis-diazotized benzidine color. Glucose was demonstrated by paper chromatography and the Glucostat reagent (Worthington Biochemical Corp.).

Examination of Lemon Peel Phenolics. Lemon peel (53 grams fresh) was ground four times in a blender with 100 ml. of 95% ethanol each time. The phenolics were extracted further from the residual pulp with 0.1*N* NaOH and finally with alcohol for 48 hours in a Soxhlet extractor. Hesperidin crystallized out of the latter solutions and was filtered and weighed. The other extracts and filtrates were evaporated to remove the alcohol and taken up in 0.1*M* acetate buffer (pH 4.6). Hemicellulase was added, and the mixture was left at room temperature for about 60 hours. The hydrolysis mixture was concentrated and worked up by the acetone precipitation method. The constituents were estimated by the same chromatographic procedures used for the juice.

Phenolic Constituents of Lemon Juice Cloud. Ten milliliters of lemon juice were filtered through Celite, and the residue was washed several times with water. The phenolics were extracted from the filter cake with 15 ml. of dimethyl sulfoxide (DMSO), in which hesperidin is very soluble. The DMSO solution was diluted with water (5% DMSO), buffered with sodium acetate–acetic acid to pH 4.6, and treated with hemicellulase. The aqueous mixture was concentrated on a rotary vacuum evaporator to remove the water, and the DMSO was removed by vacuum distillation at 50° C. The residue was taken up in acetone, and the phenolics were estimated by chromatography.

Results and Discussion

Precision and Accuracy of the Method. The standard deviation of replicate samples of standards and juice extracts was 6 to 8% of the mean. The precision was limited primarily by those factors inherent in handling small samples. Application of small sample spots uniform in size was imperative. To investigate the possible loss of phenolics in the acetone precipitations, alcoholic extracts of the precipitates were concentrated and chromatographed. Any

phenolics in the extracts were below the limit of detection of spray reagents, or less than a 1% loss.

The use of standards on each chromatogram reduced the possibility of differences in color densities owing to reagent, time, and temperature variations. Furthermore, the standard curves were obviated. The plots of densitometer areas *vs.* the amounts of the constituents were essentially linear over the range encountered in the procedure (0.5 to 2.0 μg.). This linearity permitted estimation of the juice phenolics by direct proportion. The most accurate results, however, could be obtained by selecting the amount of the standard to approximate closely that of the juice constituent.

The aglycones rather than the glycosides were used because they separated better chromatographically, and the aglycones were more readily available for standards. The aglycones were either commercially available, or in the case of eriodictyol, could be isolated easily from the peel. The enzyme mixture hemicellulase was selected because of its successful use by Horowitz and Gentili (*8*, *12*, *13*) on phenolic glycosides of lemon. However, a recent batch of the enzyme was less active, failing to hydrolyze phlorin under the experimental conditions. The situation was corrected by adding β-glucosidase (Mann Research Lab) to the mixture. The β-glycosidase could not be used alone since it would not hydrolyze eriocitrin.

Paper chromatography was chosen for its applicability to densitometry, ease of handling, availability, and permanence. Citrate buffer salts, protein, and sugars were removed by precipitation with acetone, leaving the phenolics in solution, rather than attempting to extract the phenolics quantitatively. From duplicate samples, precipitation yielded more phenolics than did six extractions with ethyl acetate.

Composition of Lemon Juice Phenolics. Identification of the constituents was based mainly on comparisons of physical, chemical, spectral, and chromatographic properties with known compounds. For years, hesperidin and an eriodictyol glycoside (*25*) have been generally recognized as constituents of lemon juice. The other phenolics cited in Figure 1, although previously known in lemon peel (*8–10*, *12–14*), have not been reported in the juice. To find the same constituents in the juice as in the peel, but in different proportions, was not surprising.

The compositional data are in Table II. Although the phenolics occurred as glycosides in the natural juice, they were reported as aglycones. Eriodictyol and hesperetin both were present as the rutinosides; phloroglucinol occurred as the glucoside. Quercetin and umbelliferone both appeared as glycosides, but their structures have not been proved unequivocally. Chopin, Roux, and Durix (*3*) suggested the presence of quercetin-3,5-diglucoside in lemon peel on the basis of spectral evidence. This appeared to be the same quercetin glucoside that the authors observed in lemon juice by paper chromatography. Additional evidence that the compound was a glucoside came from the observation that the substance was hydrolyzed by β-glucosidase.

A larger relative percentage of eriodictyol was found in coastal lemons than in desert lemons (Table II). Hesperetin showed the reverse trend. The total phenolics content of coastal fruit was also higher, on the average, than fruit from the desert (unpublished results). The Florida lemon concentrate followed the general pattern of the California coastal fruit. Determination of the source of lemons for the other commercial juice concentrates was not possible.

The effect of processing on the phenolic composition was rather striking. As previously reported (*29*), a large batch of randomized lemons was juiced commercially and finished under very light and very heavy conditions de-

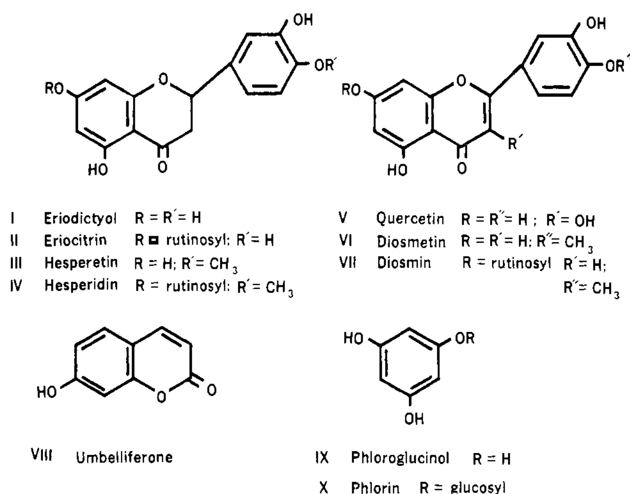

Figure 1. Phenolic compounds in lemon juice

Table II. Phenolic Content of Lemon Juice[a]

	Eriodictyol		Hesperetin		Quercetin		Phloroglucinol		Umbelliferone	
	C[b]	%[c]	C[b]	%[c]	C[b]	%[c]	C[b]	%[c]	C[b]	%[c]
Commercial concentrate	22	75	1.2	4.1	1.8	6.2	4.0	14	0.2	0.7
Commercial concentrate	16	66	1.8	7.4	3.5	14	2.7	11	0.2	0.8
Commercial concentrate	41	83	1.0	2.0	4.2	8.5	3.0	6.1	0.3	0.6
Commercial concentrate	9.4	81	1.0	8.3	0.78	6.7	0.39	3.4	0.1	0.9
Italian concentrate	22	87	1.0	4.0	0.64	2.5	1.5	6.0	0.07	0.3
Florida concentrate	12	75	1.0	6.3	1.9	12	1.0	6.3	0.05	0.3
Coastal concentrate	11	71	1.2	7.7	1.5	9.6	1.7	11	0.2	1.3
Fresh coastal	32	80	1.0	2.5	5.3	13	2.0	4.9	0.3	0.8
Fresh coastal	31	76	0.5	1.2	2.4	5.9	6.6	16	0.3	0.7
Desert concentrate	4.9	62	1.7	22	1.2	15	0	0	0.1	1.3
Fresh desert	5.7	67	1.0	12	0.74	8.7	1.0	12	0.07	0.8
Fresh desert	2.4	55	1.0	23	0.45	10	0.5	11	0.05	1.1
Processing—heavy	53	85	3.0	4.8	4.0	6.4	2.0	3.2	0.4	0.6
Processing—light	17	74	3.0	13	1.7	7.4	1.0	4.3	0.4	1.7
Average	20	74	1.4	8.5	2.2	9.0	2.0	7.8	0.2	0.9
Standard deviation		9.2		6.9		3.5		3.8		0.4

[a] All values adjusted to an average single-strength juice (citric acid: 100 meq./100 ml.).
[b] Concentration of aglycones in mg./100 ml. juice.
[c] Relative percentage of total phenolics measured.

signed to bracket commercial practice. The heaviest pressure introduced more albedo particles into the juice. Thus, the concentration of the more soluble glycosides increased, while the relatively insoluble hesperidin concentration remained constant.

The hesperetin content in all of the samples was fairly constant and was approximately equal to the solubility of the corresponding hesperidin in water of 20 p.p.m. (*25*). This solubility effect was apparent when one investigated the insoluble, suspended matter in lemon juice known as cloud. This cloud was a complex fraction containing flavonoids, sterols, carotenoids (*30*), cell fragments, and other substances. The flavonoid glycosides made up about 2% of the cloud. The flavonoid analysis of cloud from fresh, hand-reamed lemons and commercial concentrate is shown in Table III. Commercial juice cloud contained much more hesperetin than hand-reamed juice, presumably because of the pressures involved in commercial juicing and finishing techniques. No eriodictyol, quercetin, phloroglucinol, or umbelliferone was detected in the cloud. There was a second spot, however, which was chromatographically identical with diosmetin VI. This compound, when isolated by thin-layer chromatography, had UV spectra and shifts identical with authentic diosmetin. These results were consistent with the low solubility of hesperidin and diosmin VII. In Table IV are listed the amounts of the major juice phenolics in the peel. The relative percentages of the constituents were quite different for the juice and peel. In the peel, phloroglucinol was nearly equal to eridictyol, while in the juice the amount was much lower.

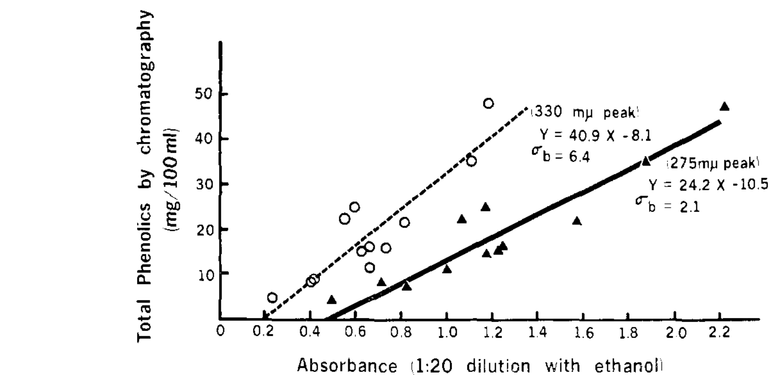

Figure 2. Correlation of absorbance and total phenolics by chromatography

Table III. Flavonoid Composition of Lemon Juice Cloud[a]

Source	Hesperetin, Mg./Cloud from 100 Ml. Lemon Juice	Diosmetin, Mg./Cloud from 100 Ml. Lemon Juice
Fresh hand reamed Yuma, Ariz. lemons	1.6	0.7
Commercial lemon concentrate	19	3

[a] Corrected to average single strength juice value (acid: 100 meq./100 ml.).

Table IV. Some Phenolic Aglycones in Lemon Peel

	Dry Weight, Mg./100 Grams Dry Peel	Relative Percentage
Eriodictyol	127	11
Hesperetin	908	78
Quercetin	32	3
Phloroglucinol	98	8

Review of the Conflicting Literature on Lemon Juice Flavanones. All of the previous literature claimed that hesperidin was the major flavanone glycoside in lemon juice (*25*). The results in Table II contradict the earlier findings by establishing eriocitrin as the major flavanone glycoside. The problem was partially one of definition. If the juice was considered to be the homogeneous liquid portion of the endocarp, then eriocitrin predominates. If, on the other hand, the juice was considered to be the heterogeneous mixture of the soluble and suspended matter, then hesperidin and eriocitrin were present in approximately equal amounts. For obvious analytical reasons, the homogeneous solution was chosen for this work. Part of the confusion arose because some of the earlier procedures actually measured eriocitrin instead of hesperidin. Furthermore, considerable confusion existed in the nomenclature of the eriodictyol glycoside. The situation merited a brief review of the literature.

The first mention of an eriodictyol glycoside in the lemon was by Bruckner and Szent-Györgyi (*2, 27*). They named the glycoside eriodictin but presented no experimental evidence for the structure. Several years later Mager (*17*) described the isolation of a crystalline eriodictyol rhamnoside eriodictin from citrin. Horowitz and Gentili (*12*) were unable to repeat Mager's results.

In 1947, Davis (*4*) developed a nonspecific, alkaline diethylene glycol method for colorimetrically estimating flavanones in citrus juices. He measured lemon juice in terms of hesperidin and stated that an eriodictyol glycoside might be present, also. His reported average, 46 mg. per 100 ml. (fresh centrifuged), was close to that calculated for the rutionosides of the average eriodictyol plus hesperetin, 44 mg. per 100 ml. in Table II. Other workers used Davis' method and reported the flavanones of the juice as hesperidin (*19*). A review of these results in 1951 by Sinclair and Bartholomew (*25*) tended to establish hesperidin in the literature as the major flavonoid in lemon juice. In 1959, Horowitz and Gentili (*11*) pointed out that Davis' method was not particularly suitable for the analysis of hesperidin.

Horowitz and Gentili (*12*) in 1960 isolated the eriodictyol glycoside and conclusively proved the structure to be eriodictyol 7-β-rutinoside (6-*O*-α-L-rahmnosyl-D-glucose). They named the compound eriocitrin to avoid confusion with the previously reported eriodictin, eriodictyol rhamnoside (*17*). By this time, however, the name eriodictin had been in use for some 24 years and was well established in the literature. In view of Horowitz and Gentili's failure to confirm Mager's results (*12*) the existence of eriodictin is subject to question.

Two years later, Hörhammer and Wagner (*7*) developed a colorimetric method for determining citrus bioflavonoids as the 2,4-dinitrophenylhydrazones. They listed (without references) hesperidin and eriodictin as present in lemon juice. However, using thin-layer chromatography and a general color reagent, they found only hesperidin. The authors observed, in this laboratory, that hesperidin and eriocitrin cochromatographed in their solvent system. The eriocitrin was detected easily by the use of the ammoniacal silver nitrate reagent.

There were several probable causes for the confusion in the earlier literature. Hesperidin, because of its insolubility, had been isolated early from lemon peel and recognized as a major constituent. Hesperidin, crystallizing out on juice extraction equipment, seemed to come from the juice, but more likely came from the cloud, albedo, and/or pulp. On the other hand, the eriocitrin, which was so soluble in water, was overlooked for many years. Even after eriocitrin was reported there was much confusion owing to nomenclature.

Applications. There was a great deal of interest in the physiological activity and value of citrus phenolics or citrus

bioflavonoids. Some of the early reports by Rusznyák and Szent-Györgyi (*24*) attempted to assign vitamin activity to the crystalline phenolic fraction from lemon juice. More recently, Martin and Szent-Györgyi (*18*) reviewed the physiological activity of the bioflavonoids. Hendrickson and Kesterson (*6*) reviewed the literature on hesperidin which also included many reports of its therapeutic activity. Some authorities (*15*) still are skeptical, however, due to the lack of well controlled clinical studies. Nevertheless, on the basis of these claims a market for citrus bioflavonoids flourishes. The authors' procedure could be very useful in assaying bioflavonoid preparations.

An earlier paper from this laboratory (*28*) established a high correlation between the citric acid content of lemon juice and the total phenolics. The measure of total phenolics in the juice was in terms of absorbance at a standard dilution. This absorbance could not be attributed to any single compound owing to the complexity of the phenolic fraction. A plot of the total phenolics, determined by paper chromatography *vs.* absorbance at both the 275 mμ peak or inflection point and the 320 to 330 mμ peak in Figure 2, showed the two methods agreed rather closely. The slope of the line for the short wavelength peak had a smaller variance than the slope for the long wavelength peak, probably because of the predominance of the eriocitrin λ_{max} at 284 mμ. Least-squares lines of the data intercept the positive absorbance axis when the phenolics are zero. The extra absorbance is probably by nonphenolic, UV-absorbing compounds.

Because of the high correlation between the total phenolics and the citric acid content in lemon juice (*28*), the total phenolics have been quite useful commercially in determining juice authenticity. However, since the phenolics were estimated in terms of absorbance, one might be tempted to add various UV-absorbing compounds to extend the juice. By these chromatographic methods, the addition of anything other than the natural mixture could be detected.

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PEANUT COMPOUNDS

Flavor Components of Roasted Peanuts. Some Low Molecular Weight Pyrazines and a Pyrrole

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Typical roasted peanut flavor is one of the most desirable and universally enjoyed flavors. However, very little is known about the compounds which constitute this unique flavor. Pickett and Holley (*9*), and more recently Young and Holley (*15*), have published information which gives some insight into the nature of some of the volatiles produced during roasting and the precursors of peanut flavor. Pattee, Beasley, and Singleton (*8*) recently reported the identities of compounds obtained from off-flavored peanuts, but there is yet to appear a definitive paper on structures of compounds which contribute to roasted peanut flavor.

This paper describes the identification of a number of nitrogenous organic compounds isolated from roasted Spanish peanuts and their possible contribution to typical roasted flavor.

Experimental

Reagents. Water, distilled and deionized.

Methylene chloride, n_D^{20}, 1.4238 Aldrich Chemical Co., Milwaukee 10, Wis., redistilled at 40.0° C.