graphic analytical method to make the qualitative statement that all the flavones listed have been found in neutral fractions throughout one entire season.

As far as the author knows, this is the first systematic attempt at analysis of such a fraction. It is hoped to devise a more practicable method of analysis and to apply it in further studies. Aside from the general qualitative and quantitative information presented in the present paper, this work led to the isolation of tetra-*O*-methylscutellarein (*5*). It is believed that this is the first isolation of this substance from natural sources, although it has long been known as a derivative of similar flavones. Tangeretin had not heretofore been known to occur in oranges. The other flavones mentioned have been found in this fruit and information concerning them has been summarized by Horowitz (*2*).

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BROWNING IN FRUITS

Nonvolatile Acids of Prunes

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Some nonvolatile acids of ripe prunes of the Italian, French, and Sweet Italian varieties were determined tentatively. Water extracts were fractionated by ion-exchange chromatography and the component acids identified on paper chromatograms. Quantitative data were obtained by titration of the acid fractions. Malic acid was the main acid of Italian and French prunes, but Sweet Italian prunes contained almost equal amounts of quinic acid. Traces of citric and, tentatively, benzoic acid were present in each variety. Phosphoric acid and possibly two isomers of chlorogenic acid were also identified.

Italian prunes (*Prunus domestica*), when harvested and marketed as fresh fruits, are commonly rejected because of a physiological disorder which has been arbitrarily called internal browning. This investigation is not related to the problem directly but was designed to be a comparative study of three varieties of prunes, in order to obtain more basic information about these fruits.

The acids of plums have been extensively studied. Prunes, however, have been studied only occasionally and a comprehensive study is not yet to be found.

Allen (*1*) observed that in plums the organic acids become localized in the flesh near the skin and around the pit, this condition becoming much more noticeable as the fruit approaches maturity on the tree.

Three isomers of chlorogenic acid were found in the prune, Imperial Epineuse, the neochlorogenic form being the most abundant, but caffeic acid was not detected (*18*). Mrak (*12*), however, found no chlorogenic acid but 0.30% caffeic acid in dried prunes. The presence of quinic acid (1%) and traces of benzoic acid have also been reported (*9*).

Procedure

Ripe fruits of Italian, French, and Sweet Italian prunes were harvested in 1963. Samples were destoned, chopped, and frozen. Later, each sample was allowed to thaw and the organic acids were extracted from 100 grams of tissue according to the methods of Dostal (*5*) and Markakis (*10*). No attempt was made to determine oxalic acid. Aliquots of each extract were titrated with standard NaOH to determine the acid content.

For fractionation, an aliquot equivalent to 1 meq. of the extract was put through a 20 × 0.7 cm. column of Dowex 1-X8 resin (100- to 200-mesh, acetate form) followed by 10 ml. of water. The gradient elution system consisted of two acid reservoirs connected to a 150-ml. mixing flask by a three-way stopcock. Nitrogen gas, at a pressure of 5 p.s.i., was used to maintain a steady rate of flow, and a magnetic stirrer ensured thorough mixing. The initial eluent was 75 ml. of 4.5*N* acetic acid, followed by 150 ml. of 8*N* acetic acid, 50 ml. of 8*N* formic acid, and 70 ml. of 10*N* formic acid. Several other elution systems were experimented with, but these concentrations gave the best resolution of our extracts. Sixty fractions of 5.7 ml. each were collected, using an automatic fraction collector.

The fractions were evaporated to dryness in vacuo at 40° C. and dissolved in 1 ml. of hot water. One milliliter of 0.002*N* NaOH, containing 0.01% phenolphthalein indicator, was added to each tube, using an automatic pipet (*6*). This served as a base line for titration, and those fractions which remained colorless (indicating the presence of acid) were titrated to the phenolphthalein end point with 0.01*N* NaOH. After titration, the fractions were dried in vacuo at 40° C. for qualitative determination by paper chromatography. The residues were dissolved in 0.5 ml. of 50% ethanol and 0.1 ml. of an aqueous slurry of Dowex 50-X8 resin (H^+ form, 50- to 100-mesh) was added to each fraction to remove excess sodium ions. The dissolved fractions were spotted on Whatman No. 1 16- × 46-cm. sheets, 2 cm. apart, and 5 cm. from the short edge of the sheet. The papers were held in a chromatography cabinet for

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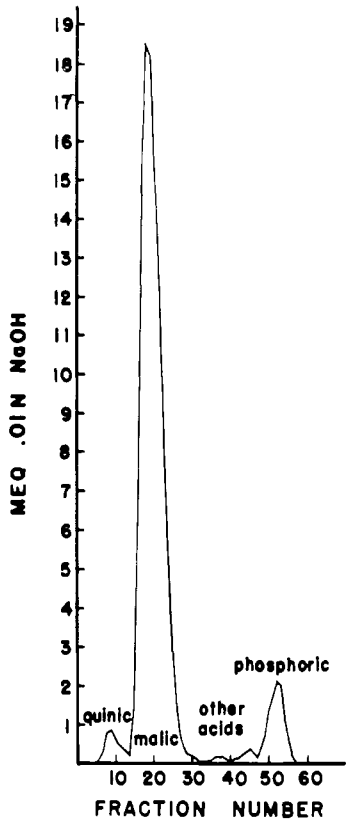

Figure 1. Acid profile of Italian prune

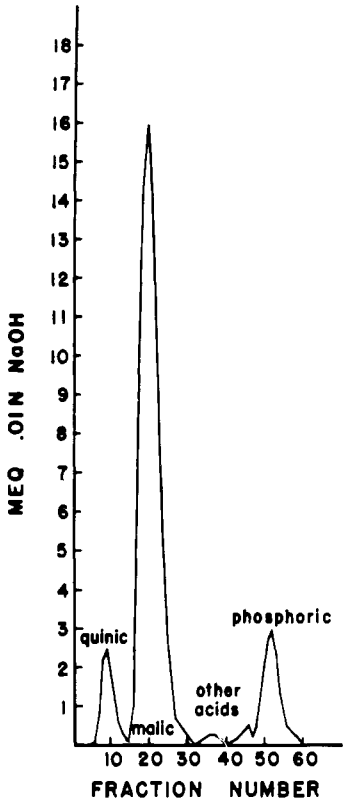

Figure 2. Acid profile of French prune

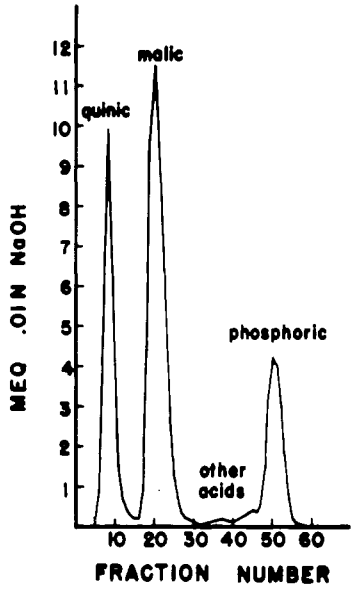

Figure 3. Acid profile of Sweet Italian prune

4 hours in an atmosphere generated by the lower phase of the solvent system—viz., 1-butanol and 3*N* formic acid, 50:50 by volume. The papers were then irrigated descendingly for ca. 12 hours at 20° C., using the upper phase. After drying in an air stream for 2 hours, the papers were sprayed with a combined detecting reagent (*16*). The papers were examined under ultraviolet light for further characterization of chlorogenic and caffeic acids.

To confirm the order of elution and the per cent recovery of acids identified in the extracts, commercial preparations of these acids in solution were fractionated *en masse* and individually in quantities representing their concentrations in the extract. Recovery exceeded 90% in all cases except quinic acid.

Table I. R_f Values for Organic Acids of Prune Fruits Separated by Ion-Exchange Chromatography and Spotted on Paper Chromatograms beside Known Acids

Acid	R_f Known	R_f Unknown
Quinic	0.22	0.22
Malic	0.55–0.56	0.55–0.56
Chlorogenic	0.65–0.70	0.65–0.70
Citric	0.48–0.49	0.48–0.49
Benzoic	0.93–0.94	0.90–0.94
Caffeic	0.79–0.81	...
Phosphoric	0.35–0.38	0.34–0.36
Chlorogenic acid isomers		0.56–0.62
		0.80–0.81

These known acids were chromatographed on paper after elution from the column. These chromatograms and those from the cochromatography of unknown acids were used to compile identification data.

Results and Discussion

Figures 1, 2, and 3 are the acid profiles of Italian, French, and Sweet Italian prunes. The evidence from paper chromatography is that the minor peaks between malic and phosphoric acids are made up of chlorogenic acids and traces of citric, benzoic, and probably caffeic acids. Fractionation of a standard solution of these acids confirmed that their elution from the resin column is not discrete. Because these components are present in trace amounts, they are grouped as "other acids" for quantitative treatment.

The R_f values of known acids and those present in the extracts are given in Table I. Three spots were obtained which fluoresced under ultraviolet light: One corresponded to caffeic acid and another to chlorogenic acid; the third had a lesser R_f than either of the first two and is assumed to be a chlorogenic acid isomer. Caffeic acid has been identified in dried prunes only (*12*) but never in fresh plums or prunes; neochlorogenic, chlorogenic, and isochlorogenic acids have all been identified (*4*, *18*) as present in prunes. It is possible, therefore, that the three spots on our chromatograms are chlorogenic acid isomers. Unfortunately, the iso- and neo- forms of chlorogenic acid were not immediately available for confirmatory work. However, the occurrence of caffeic acid as an artifact in the extraction for fractionation procedures cannot be discounted, inasmuch as chlorogenic acid, which is a depside of quinic and caffeic acids, might have undergone slight hydrolysis. The identification of benzoic acid is tentative. However, this acid has been found in prunes (*9*) and Miller (*11*) reports that prunes are relatively rich in benzoic acid.

Table II shows the total acidity and the amount of quinic, malic, phosphoric, and other acids in Italian, French, and Sweet Italian prunes. The Sweet Italian prunes contained a greater amount of quinic acid. It is presumed that this is a varietal characteristic. An

Table II. Total Acidity and Composition of Prunes

(Meq. per 100 grams fresh weight)

Variety	Total Acidity	Quinic	Malic	Phosphoric	Other Acids	Total	% Recovery of Total Acidity
Italian	12.43	0.40	10.10	0.93	0.23	11.66	93.8
French	8.68	0.56	6.23	0.89	0.25	7.93	91.4
Sweet Italian	6.73	1.49	2.27	0.98	0.20	4.94	73.4

inverse relationship appears to exist between the per cent recovery of quinic acid and the amount of this acid in the extract—for example, the total per cent recovery from Sweet Italian prunes is low. By fractionating solutions containing various amounts of quinic acid alone, and quinic, malic, and other acids together, it was found that, with quinic acid at a concentration approximating that in Sweet Italian prunes, 80% or less of the quinic acid is recovered. This low rate of recovery is likewise evident in French prunes, where the quinic acid content is slightly higher than in Italian prunes.

The predominant acid of Italian and French prunes is malic acid. Many studies of plums have shown this to be so. However, in the Sweet Italian prune there is almost as much quinic as malic acid. Quantities of phosphoric and other acids are about the same in all three varieties. Citric acid in small amounts has been found in plums (*3, 17*), but Dickinson and Gawler (*4*) found none in the Victoria plum. Some workers (*8, 13, 14*) have detected tartaric acid, but others (*4, 15*) failed to do so. Reports of the presence of oxalic acid (*3, 8*), glyoxalic acid (*2*), malonic acid (*8*), and salicylic acid (*19*) are to be found in the literature, but these acids were not detected in studies on the Victoria plum (*4, 15*). Differences in variety, stage of maturity, and methods of analysis must be borne in mind when these reports are considered.

No definite conclusions regarding the internal browning of prunes can be drawn from this study, but the differences in levels of quinic acid between the Sweet Italian and French varieties is striking. Internal browning has not been reported in Sweet Italian prunes. Both the Italian (*20*) and French varieties (*7*) are susceptible to internal browning; however, the disorder was not observed in any of our samples. Interest in chlorogenic acid has centered on its function as a substrate for polyphenolases; the darkening of fruits on injury may be due to the oxidation of chlorogenic acid. It is not known whether the internal browning of prunes can be explained in this way, but in a study now under way of Italian prune fruits at progressive stages of development, quinic, malic, phosphoric, and traces of citric and benzoic acids, but no chlorogenic acid, have been detected.

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AMINO ACID DETERMINATION

Microdetermination of Lysine in Protein Hydrolyzates

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A simple colorimetric procedure for the direct microdetermination of lysine in protein hydrolyzates is based primarily on quantitative conversion of the amino acids into their copper salts, followed by treatment of the latter with 1-fluoro-2,4-dinitrobenzene. Under these conditions, lysine will be the only amino acid which yields a colored dinitrophenyl derivative, and it can be estimated by measuring the absorbance of the copper-free and ether-extracted mixture. The accuracy and specificity of the method have been demonstrated. The procedure has been applied to micro quantities of proteins, and the results are comparable with those previously reported. The present method is direct and economical in time and materials, and does not require special equipment.

THE early chemical methods for determination of lysine in protein hydrolyzates are indirect, tedious, and nonspecific. These difficulties are partly overcome by the microbiological and enzymic assays, which, however, are time-consuming. The defects inherent in the older methods are avoided by the use of ion exchange chromatography. The new procedures are far from simple, and at each step require careful attention to specific conditions.

A satisfactory chemical procedure which may be applied specifically for the estimation of lysine in protein hydrolyzates has not been developed previously. The determination of a specific amino acid such as lysine is of value in nutrition and metabolic studies. The present investigation has been undertaken to develop a simple chemical micromethod