Characterization of Prune Juice

H. van Gorsel, ChingYing Li, Eduardo L. Kerbel[1], and Adel A. Kader*

Department of Pomology, University of California, Davis, CA 95616

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[1] Present address: Department of Horticulture, Plant Sciences Laboratory, 1201 South Dorner Dr., University of Illinois, Urbana, Il 61801.
* To whom correspondence should be addressed.

Abstract

Juice of prune and nine other fruits was analyzed by HPLC for amino acids, anthocyanins, phenolic compounds, organic acids and sugars. Prune juice can be identified by the absence of anthocyanins, the predominance of quinic acid and the absence of citric and tartaric acids. Sorbitol content in the fruit is in the same concentration range as glucose and fructose, while the concentration of sucrose is low. The phenols (-)-epicatechin and phloridzin are absent from prune juice. Consequently, measurement of individual organic acids and phenylpropanoids is sufficient to distinguish prune juice from any of the other fruits tested (apple, cherry, grape, kiwifruit, nectarine, peach, pear, plum and strawberry).

Introduction

Prune juice is a valued product that commands a high price on the market. Production of prune juice from dried prunes is expensive. Therefore there is an economic incentive for adulteration of prune juice with cheaper fruit juices, fruit juice concentrates and/or sugar syrups.

To determine the authenticity of prune juice a comprehensive knowledge of its composition is required. Various authors (Flynn and Wendt, 1970; Henning and Herrmann, 1980; Ishii, 1983; Möller and Herrmann, 1983; and Mosel and Herrmann, 1974) have investigated some aspects of prune juice composition, However, little information is available on organic acids, anthocyanins and free amino acid composition of prune juice; and comparisons of fruit juice composition data among reports are often complicated by differences in analytical methods used (Reyes *et al.*, 1982).

Therefore, our objective was to analyze juice of prunes and nine other fruits for sugars, organic acids, free amino acids, phenolic compounds and anthocyanins; and to identify those components that can be used as markers for establishing the authenticity of prune juice.

Materials

Source of Fruits. 'Red Delicious' apples, 'Bing' cherries, 'Black Seedless' grapes, 'Hayward' kiwifruits, 'Red Delight' nectarines, 'Desert Gold' peaches, 'd'Anjou' pears, 'Roysum' plums and 'Douglas' strawberries were bought from a wholesale market (General Produce, Sacramento, CA). Where possible fresh fruits were used for the analyses. Otherwise, they were frozen at -30 °C (for one to three months). Unless otherwise indicated, for each species three replicates consisting of ten fruits each were used for each of the analyses. A commercial prune juice ("Sun Sweet" brand) was purchased locally. The juice was kept at 4 °C and aliquots were drawn as needed. Sucrose, corn syrup and high-fructose corn syrup (HFCS) were used in some of the analyses for comparison.

Apparatus. A BioRad HPLC system equiped with UV monitor and refractive index (RI) detector was used for the analysis of organic acids, sugars and individual phenolics. Mobile phases were continuously degassed with helium. Anthocyanins were analyzed with a Hewlett Packard HPLC system with diode-array detector. Amino acids were analyzed with a Beckman model 7000 amino acid analyzer with dual channel spectrophotometric detector. In all cases tentative identification of constituents was based on comparison of retention times with those of known standards.

Procedures

Determinations of Sugars and Organic Acids. Fruit juice was prepared by squeezing wedges of 5 or 10 whole fresh or frozen fruits through 2 layers of cheesecloth (Hamilton Beach fruit press). The juice was centrifuged at 25,000 g for 25 minutes at 4 °C. The supernantant was adjusted to pH 8-9 with 58% NH_4OH. Two ml of this solution was passed through a column with 2 g anion exchange resin (Bio-Rex5 analytical grade, 100-200 mesh, chloride form). Subsequently the column was rinsed with 8 ml double deionized water. This eluate was collected for sugar analysis. Next, 2 ml of 10% H_2SO_4 was added to the column, followed by rinsing the column with 8 ml of double deionized water. The eluate was collected for organic acid analysis.

Individual sugars were separated with a 25 x 0.4 cm HPX-87C column at 85 °C and detected with a RI monitor. Double deionized water was used as the mobile phase at a flow rate of 0.6 ml/min. Known standards were: sucrose, glucose, fructose and sorbitol.

Individual organic acids were separated with a 25 x 0.4 cm HPX-87H column at 65 °C for citric, malic and quinic acids and at 24 °C for tartaric and ascorbic acids. Mobile phases were: 0.05 N H_2SO_4 at 0.8 ml/min for citric malic and quinic acids; 0.002 N H_2SO_4 at 0.6 ml/min for tartaric acid; and 0.01 N H_2SO_4 at 0.5 ml/min for ascorbic acid. The RI monitor was use for determining citric, malic, quinic and tartaric acids. Ascorbic acid was determined with a UV monitor at 245 nm. Known standards were: citric, malic, quinic, tartaric and ascorbic acids.

Determination of Phenolic Compounds. Ten grams of flesh tissue from 5 or 10 fresh or frozen fruits were homogenized with 20 ml of 100% methanol in a Polytron homogenizer. The homogenate was filtered through Wattman No. 4 filter paper. The filtrate was evaporated to dryness in 10 to 11 hours with a Speed Vacuum Concentrator. The residue was dissolved in 7 ml 100% methanol. For prune juice, 10 ml juice was directly evaporated to dryness in 5.5 hours with a Speed Vacuum Concentrator. The residue was dissolved in 20 ml methanol to extract the phenolics. The extract was filtered through Wattman No. 4 filter paper. The filtrate was concentrated to 7 ml by a Speed Vacuum Concentrator. All samples were kept in a freezer overnight.

The next day, 1 ml extract was diluted with 4 ml of 0.1 M $NH_4H_2PO_4$-buffer (pH 2.8). Phenolic compounds were separated with a 25 x 0.4 cm reverse phase C-18 column (Bio-Sil ODS-5S) and detected with an UV monitor at 280 nm; 0.1 M $NH_4H_2PO_4$-buffer (pH 2.8) ('A') and 80% methanol ('B') were used as mobile phases. The changes in the mobile phases were modified from the method reported by Wulf and Nagel (1976). After 2 initial minutes at 20% B the gradient was 20 to 80% B in 12 minutes, kept at 80% B for 10 minutes, and returned to 20%B in 5 minutes. The flow rate was 0.6 ml/min. Known standards were: (+)-catechin, chlorogenic acid, (-)-epicatechin, caffeic acid, ferulic acid and phloridzin. Standards were prepared in 100% methanol and then diluted with 0.1M $NH_4H_2PO_4$-buffer pH 2.8 (1:5, v/v).

Determination of anthocyanins. Ten gram of flesh tissue were homogenized with 15 ml of 100% methanol (HPLC grade) in a Polytron homogenizer at intermediate speed. The homogenate was centrifuged at 25,000 g for 25 minutes at 4 °C. The supernatant was made up to 25 ml with 100% methanol (HPLC grade). Individual anthocyanins were separated with a 25 x 0.4 cm Hibar LiChrosorb RP-18 (5 micron) reversed phase column. Eluent was monitored at 326 and 520 nm. At each peak an absorbance spectrum (250-600 nm) was acquired. The solvent method of Wulf and Nagel (1978) was used with the following modifications: Mobile phase A: 10% formic acid; mobile phase B: 10% formic acid in 50% acetonitrile. The initial condition of 25% B for 20 min. was followed by an increase to 75% B in 10 min. This condition was kept for 5 min. before returing to the initial condition. Flowrate was constant at 1.0 ml/min.

Determination of fee amino acids. Fruit juices were prepared as for sugars and organic acids, then 1:5 and 1:50 dilutions were frozen until used for analysis. Lithium buffers were used at a flowrate of 20 ml/hr. The eluent was monitored at 420 and 700 nm.

Results and Discussion

Organic acids. Organic acid composition of fruits is strongly influenced by fruit maturity, geographic origin and climatic conditions, as was shown for peach and pear (Wrolstad *et al.*, 1981) and strawberry (Reyes *et al.*, 1982) fruits. Nevertheless, organic acid analysis can give useful information.

Compared to all other fruit juices, prune juice is relatively low in malic acid, but its quinic acid concentration matches that of kiwifruit (table 1). Prune juice contains very little vitamin C and no detectable amounts of citric acid. In contrast, strawberry, kiwifruit, peach and nectarine all contain large amounts of citric acid.

Tartaric acid is absent in all fruits except grape juice. Tartaric acid can, however, be crystallized out and is therefore not a reliable marker for adulteration with grape juice.

On the basis of these organic acid analyses only mixing prune juice with pear and plum juice is difficult ot detect.

Addition of organic acids to diluted prune juice will change the organic acid profile. If commercial malic acid is used, the L-malic to total malic acid ratio will be less than 1.

Sugars. Prune juice is high in sorbitol and low in sucrose content (table 2). Similarly, Kline and Fernandez-Florez (1970) found only small amounts of sucrose in juice of 'Imperial' and French prunes and no sucrose in California or 'Robe de Sergent' processed prunes.

Prunes contain invertase (Wrolstad and Shallenberger, 1981) which seems to be responsible for the disappearance of sucrose during processing. Flynn and Wendt (1970) have used the presence of sucrose as an indicator of adulteration.

Prune juice has a glucose to fructose ratio of approximately 1.5. Peach and nectarine also have a glucose to fructose ratio of greater than one, but contained much more sucrose, in agreement with Eheart and Mason (1967).

In contrast, apple and pear have much more fructose than glucose. The other fruits appear to have an invert sugar pofile. Similar results were reported by Flynn and Wendt (1970), Wrolstad *et al.* (1981) and Wrolstad and Shallenberger (1981).

Consequently mixing prune juice with most of the other fruits or with corn syrup, high fructose corn syrup (HFCS) or (inverted) beetsugar will cause clear changes in the sugar profile. Addition of HFCS or corn syrup can be confirmed by stable isotope ratio determination.

Natural phenols. The presence and amount of six common natural phenols are shown in figure 1. Chlorogenic acid and (+)-catechin were present in all fruits, except kiwifruit which contained (+)-catechin only. Phloridzin was found only in apple and pear juice. The absence of (-)-epicatechin distinguished prune juice from pome fruits and the other stone fruits, except peach. With respect to the six phenols for which we assayed, strawberries showed only quantitative differences compared to prunes. Grapes had a very different and complex phenolic composition compared to any of the

other fruits (data not shown). Our results are in general agreement with the findings of van Buren (1970), Mösel and Herrmann (1974) and Möller and Herrmann (1983). The latter authors also identified neochlorogenic acid and 3'-p-coumaroyl quinic acid in some pome and stone fruits. However neither of these phenols could account for the still unknown predominant peak (peakheight 532 to 544) with a concentration range of 100 to 450 mg per 100 ml prune juice, depending on molar absorbance.

Free amino acids. Alpha-amino butyric acid, citrulline, cystathione, o-phosphoethanolamine, phosphoserine and taurine were most characteristic of prune juice, and did not occur in significant amounts in any of the other fruits tested for these amino acids (table 3). Prune juice had approximately three times more gamma-amino butyric acid than any of the other stone fruits, but was much lower in cysteine and glutamine content. Pear juice contains a lot more cysteine than prune juice. Cherry fruit is relatively high in tryptophan. Both kiwifruit and grapes had a lot more arginine, cysteine, methionine and tryptopan than prunes. Grapes also had more alanine, proline and valine. These latter differences, and the increased amounts of serine in strawberry and lysine in kiwifruit were not observed by Fernandez-Florez *et al.* (1970). This indicates that care has to be taken in drawing conclusions from quantitative differences in free amino acid content. Variety, cultural practices, season, maturity at harvest and storage conditions could all affect the pool of free amino acids.

Anthocyanins. Only prune, pear and kiwifruit did not have detectable amounts of anthocyanins. Therefore presence of anthocyanins in prune juice is a clear mark of adulteration. The anthocyanin composition of other fruits is shown in table 4. The identification of the anthocyanins was based on relative retention-times (Williams *et al.*, 1978, Wulf and Nagel, 1978) and absorbance spectra (Hebrero *et al.*, 1988). Complexation of phenolic compounds and/or anthocyanins probably is responsible for the brown color of prune juice. Indications for this possibility are that fresh plums contain cyanidin-3-glucoside and various phenols, and that the estimated amount of total anthocyanins in grape juice (Lamikanra, 1988) is about the same as the amount of unknown phenol in prune juice.

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Table 1. Determination of Organic Acids in Various Fruit

Fruit	Organic Acid (mg/100 ml Juice)				
	Citric	Ascorbic	Malic	Quinic	Tartaric
Apple	ND	Tr	518 ± 32	ND	ND
Cherry	ND	Tr	727 ± 20	ND	ND
Grape	Tr[a]	Tr	285 ± 58[b]	ND[c]	162 ± 24
Kiwifruit	730 ± 92	114 ± 6	501 ± 42	774 ± 57	Tr
Nectarine	140 ± 39	Tr	383 ± 67	136 ± 28	ND
Peach	109 ± 16	Tr	358 ± 72	121 ± 11	Tr
Pear	ND	Tr	371 ± 16	220 ± 2	ND
Plum	ND	Tr	294 ± 24	214 ± 68	ND
Prune	ND	Tr	104 ± 14	668 ± 25	ND
Strawberry	207 ± 35	56 ± 4	199 ± 26	ND	ND

[a] Tr = Trace

[b] Mean ± standard Deviation, n = 3

[c] ND = Nondetectable

Table 2. Determination of Sugars in Various Fruits

Fruit	Sugar (g / 100 ml Juice)			
	Sucrose	Glucose	Fructose	Sorbitol
Fruit				
Apple	0.82 ± 0.13[a]	2.14 ± 0.43	5.31 ± 0.94	0.20 ± 0.04
Cherry	0.08 ± 0.02	7.50 ± 0.81	6.83 ± 0.74	2.95 ± 0.33
Grape	0.29 ± 0.08	9.59 ± 1.03	10.53 ± 1.04	ND[b]
Kiwifruit	1.81 ± 0.72	6.94 ± 2.85	8.24 ± 3.43	ND
Nectarine	8.38 ± 0.73	0.85 ± 0.04	0.59 ± 0.02	0.27 ± 0.04
Peach	5.68 ± 0.52	0.67 ± 0.06	0.49 ± 0.01	0.09 ±0.02
Pear	0.55 ± 0.12	1.68 ± 0.36	8.12 ± 1.56	4.08 ± 0.79
Plum	0.51 ± 0.36	4.28 ± 1.18	4.86 ± 1.30	6.29 ± 1.97
Prune	0.11 ± 0.03	7.00 ± 0.31	4.63 ± 0.29	4.35 ± 0.24
Strawberry	0.17 ± 0.06	1.80 ± 0.16	2.18 ± 0.19	ND
Syrup				
Sucrose (66.5%)	61.80 ± 3.60	ND	ND	ND
High Fructose (71.0%)	3.37 ± 0.06	32.60 ± 0.56	32.30 ± 0.25	ND
Corn Syrup	35.00 ± 4.24	28.90 ± 3.18	ND	ND

[a]Mean ± standard deviation, n = 3

[b]ND = Nondetectable

TABLE 3. AMOUNT OF FREE AMINO ACIDS IN SOME FRUIT JUICES (in mg per liter juice)

	PRUNE		PEACH		PLUM		CHERRY		NECTARINE		PEAR		STRAWBERRY		APPLE		GRAPE		KIWIFRUIT	
Amino acid	avg	std	avg	std	avg	std	avg	std	avg	std	avg	std	avg	std	avg	std	avg	std	avg	std
o-phospo-L-serine	23.9	5.5	2.6	0.4	3.1	0.1	12.6	0.8	4.9	1.2										
taurine	110.4	21.6	nr		0.0	0.0	0.0	0.0	nr											
o-phosphoethanolamine	79.4	5.2	1.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0										
aspartic acid	525.7	*	352.7	23.7	359.4	42.5	195.3	8.3	516.8	58.2	128.3	2.5	113.7	5.5	155.3	29.5	77.3	9.5	95.6	1.9
hydroxyproline	nr		7.7	0.8	1.7	0.1	2.1	0.4	6.0	1.0										
threonine	35.5	4.8	85.4	12.3	63.5	5.6	24.1	3.6	95.3	21.2	6.4	1.3	51.6	1.2	3.5	0.5	54.8	1.7	37.4	3.0
serine	35.9	4.0	182.1	20.3	147.1	8.6	26.5	3.5	157.6	11.3	24.6	3.9	106.9	2.2	16.6	10.6	56.5	2.6	26.8	2.8
glutamine	3.4	*	223.2	47.5	55.1	3.1	168.1	17.8	207.1	70.7										
alpha-amino adipic acid	3.7	0.2	5.6	1.3	9.8	2.7	9.8	1.0	0.0	0.0										
proline	164.9	36.5	51.6	3.8	475.8	86.7	222.4	47.2	85.0	9.6	11.7	6.4	3.1	0.2	1.6	0.2	1006.6	32.0	19.2	1.3
glycine	4.3	0.2	15.5	2.9	14.0	2.0	3.4	0.2	14.1	1.3	1.1	0.2	8.6	0.5	1.2	0.1	4.0	0.2	9.2	0.5
alanine	59.0	2.5	131.9	22.3	197.5	17.2	6.9	0.5	103.5	15.5	7.8	1.0	143.9	8.3	14.2	0.2	172.2	16.6	35.5	0.4
citrulline	36.8	4.7	0.0	0.0	0.0	0.0	6.5	0.9	14.9	*										
alpha-amino-n-butyric acid	39.4	1.0	1.2	0.9	0.9	0.8	0.0	0.0	0.2	0.0	0.2	0.1	2.9	0.2	0.8	0.6	1.0	0.7	2.5	0.1
valine	17.3	3.9	42.2	4.7	40.7	1.6	4.3	0.2	29.9	5.4	14.6	2.1	17.7	0.9	4.7	1.2	46.7	9.3	27.3	2.5
cysteine	3.8	0.2	52.9	7.0	54.8	4.1	89.6	3.8	86.0	8.4	38.0	2.4	0.0	0.0	1.4	0.2	22.8	0.7	46.4	1.9
methionine	0.4	0.0	0.6	0.0	0.4	0.1	0.1	0.0	0.3	*	0.0	0.0	1.8	0.1	0.3	0.0	11.6	2.8	6.0	0.9
cystathione	8.9	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0										
iso-leucine	14.0	0.7	29.8	3.1	18.8	1.0	0.0	0.0	18.8	4.6	12.7	2.1	7.0	0.5	15.7	2.0	33.3	10.8	19.2	2.0
leucine	24.5	4.9	14.2	1.0	10.4	1.0	20.6	2.9	4.7	0.4	3.8	0.9	3.3	0.1	1.4	0.1	51.0	11.5	37.9	2.5
tyrosine	11.8	0.2	9.1	0.7	4.3	0.2	1.1	0.4	4.3	0.2	0.7	0.0	3.6	0.2	0.0	0.0	25.7	3.6	11.2	0.9
phenylalanine	31.1	1.7	27.3	3.8	26.8	2.3	1.3	0.2	14.5	1.0	5.0	0.2	2.5	0.2	1.3	0.2	20.8	3.5	25.8	1.8
beta-alanine	2.7	0.3	6.7	0.7	4.1	1.0	0.9	*	5.3	0.4										
gamma-amino-n-butyric acid	101.3	10.3	37.4	3.1	32.5	9.0	37.1	4.6	39.0	4.3										
ethanolamine	3.2	1.2	nr		3.7	0.5	0.0	0.0	1.2	0.2										
tryptophan	7.1	0.8	6.9	2.5	8.0	1.4	61.9	4.9	14.3	*	8.0	2.2	18.2	0.8	3.5	2.2	44.9	3.5	34.1	1.4
ornithine	25.1	15.6	46.7	10.4	27.5	7.4	10.8	0.3	13.2	0.5										
lysine	1.9	0.1	2.8	0.0	2.2	0.0	0.9	0.1	1.6	0.4	1.5	0.3	1.3	0.0	1.0	0.1	4.4	0.3	19.3	0.7
1-methyl-L-histidine	24.5	*	25.7	2.0	30.1	2.7	0.0	0.0	0.0	0.0										
histidine	14.3	0.2	27.6	3.4	17.8	1.7	39.9	5.4	34.4	7.0	11.0	3.9	15.2	3.3	11.8	0.3	50.4	2.0	41.1	2.3
arginine	1.7	0.2	1.2	0.2	1.0	0.0	2.4	0.3	0.7	0.0	0.3	0.0	7.5	3.1	1.0	0.2	591.9	14.5	49.6	8.2

blank (in avg. column) '= not determined
nr '= not resolvable
* '= one replicate only

note: aspartamine and glutamic acid were not separable with the system used

Table 4. Determination of Anthocyanins in Various Fruits

Fruit	Anthocyanin[a]	
	Identification[b]	Peak Area[c]
Apple	Cy-3-galactoside	260 ± 69
	Cy-7-ara or Cy-3-glu	27 ± 15
	Cy-3-arabinoside	22 ± 6
Cherry	Cy-3-rutinoside	1320 ± 109
	Cy-3-glucoside	189 ± 40
	Pn-3- rutinoside	47 ± 36
Grape	Mv-3-glucoside	2157 ± 375
	Dp-3-glucoside	586 ± 110
	Pn-3-glucoside	478 ± 92
	Cy-3-glucoside	121 ± 33
Nectarine	Cy-3-glucoside	322 ± 51
Peach	Cy-3-glucoside	180 ± 43
Plum	Cy-3-glucoside	42 ± 5
Strawberry	Pg-3-glucoside	1302 ± 29
	Pg-3-glycoside	78 ± 9
	Cy-3-glucoside	70 ± 18

[a] Abbreviations used in the table: Dp = Delphinidin; Cy = cyanidin; Pt = petunidin; Pg = pelargonidin; Pn = peonidin; Mv = malvidin.

[b] Tentative identification based on retention ti mes and spectral characteristics. Results were compared with: Gross (1987); Hebrero et al. (1988); Hrazdina (1982); Timberlake (1980); Timberlake and Bridle (1982); Van Buren (1970).

[c] Mean ± standard deviation, n = 3

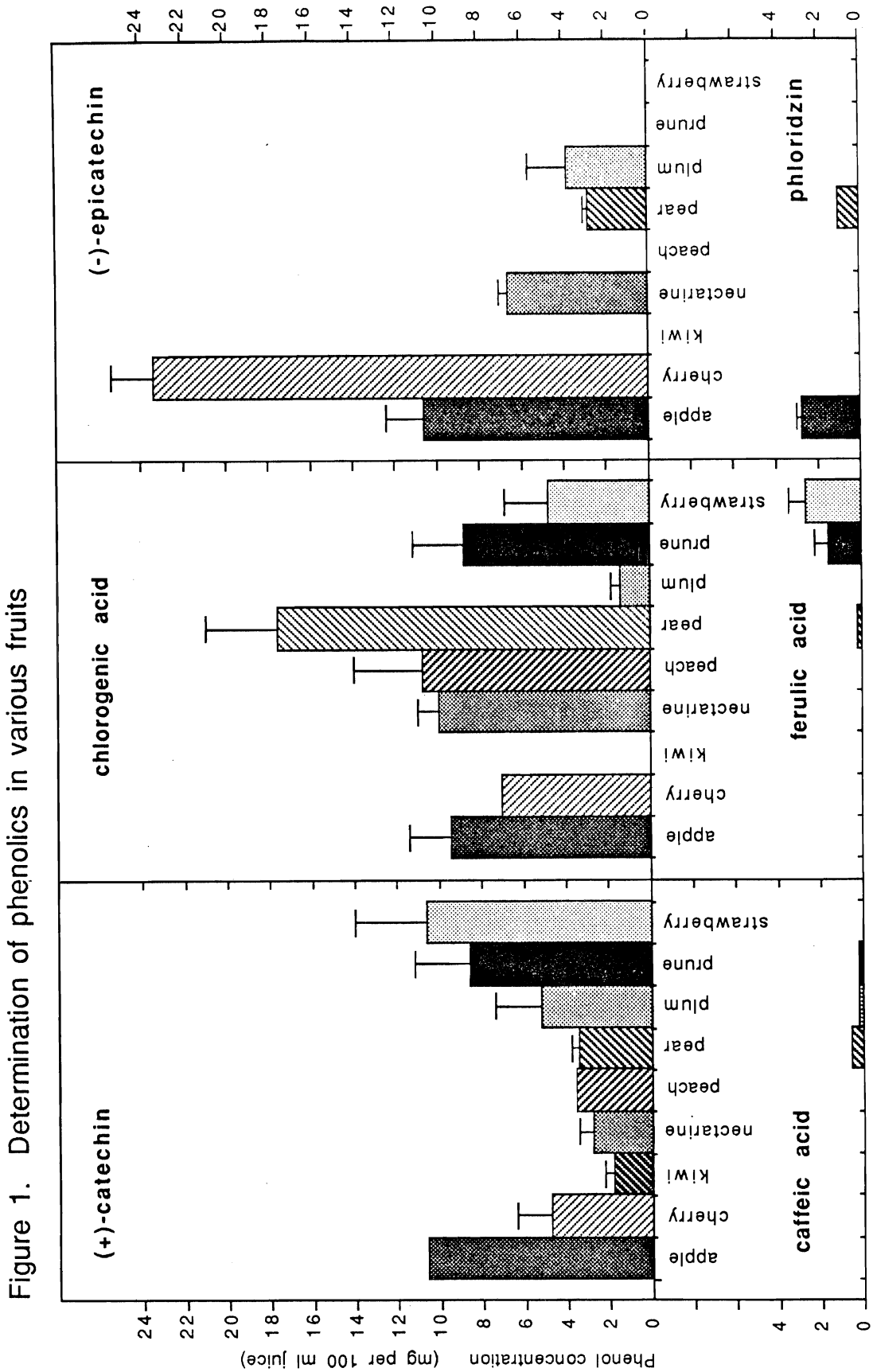

Figure 1. Determination of phenolics in various fruits