Differentiation of Citrus Juices by Factorial Discriminant Analysis Using Liquid Chromatography of Flavanone Glycosides

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Flavanone glycosides (FG) found in citrus juices have been separated and quantitatively determined by reversed-phase liquid chromatography (LC) using a C_{18} packed column and water–acetonitrile–tetrahydrofuran–glacial acetic acid eluent system. The influence of juice pH on hesperidin and narirutin determinations, from commercial orange juices, has been studied at different pH values (from 2.0 to 6.2). The response factors, averages, relative standard deviations, and recoveries of six FG were determined using LC with an UV detector at 280 nm. The six FG and three unknown compounds found in citrus juices were determined in 124 samples of lemons, limes, grapefruits, and sweet oranges. The major FG in each citrus group were as follows: in lemon and lime, eriocitrin (47–94 mg L^{-1}) and hesperidin (84–196 mg L^{-1}); in sweet orange, narirutin (30–84 mg L^{-1}) and hesperidin (235–407 mg L^{-1}); in grapefruit, narirutin (33–161 mg L^{-1}) and naringin (113–481 mg L^{-1}). Factorial discriminant analysis of the data obtained effectively differentiated lemon and lime and varieties of grapefruits (white, pink, red, and green) and sweet oranges (Valencia, navel, blood, Thomson, and Malta).

INTRODUCTION

Flavonoid compounds are widespread in the plant kingdom. Flavanone glycosides (FG) have a more restricted distribution and are specific of citrus juices (Attaway et al., 1972; Harborne et al., 1975). Among these compounds (Figure 1), naringin and neohesperidin are important with regard to quality control and bitterness of grapefruit juices (Davis, 1947; Fisher et al., 1966). The resolution and determination of these two compounds have been achieved by reversed-phase liquid chromatography (LC) (Fisher and Wheaton, 1976; Rouseff et al., 1987; Rouseff, 1988b). Two other FG, hesperidin and narirutin (Figure 1), have been determined in common sweet oranges (Kamiya et al., 1979; Rouseff, 1980; Smolensky and Vandercook, 1982). A gradient LC procedure has been developed by Rouseff (1988a) to cleanly separate and quantify flavanone glycosides in citrus juices from various cultivars comprising six common species. To differentiate some common species, additional information such as PMF concentration (especially tangeretin, i.e., 4′,5,6,7,8-pentamethoxyflavone) is necessary (Rouseff, 1988a). Eriocitrin and neoeriocitrin are generally found in large amount in lemon juices (Kamiya et al., 1979) and in sour oranges (Reminiac et al., 1989) respectively. Recently we have obtained the separation of these six FG and applied this method in grapefruit and sour orange juice adulterations (Mouly et al., 1993).

The purpose of this paper is to report a method for the determination of the FG mentioned above and generally found in citrus juices. This method used reversed-phase

Table 1. Origins and Varieties of Common Citrus Samples Investigated

common Citrus name	variety[a]	origin	n[b]
lemon		Spain	13
		France	4
lime		Brazil	8
		Mexico	6
grapefruit	white	Israel	9
		Cuba	2
	pink	Florida	14
	red	Spain	6
		Israel	3
	green	Israel	8
sweet orange	Valencia	Morocco	3
		Florida	3
		Spain	2
		Brazil	3
	navel	Spain	12
	blood	Italy	7
		Spain	2
	Thomson	Spain	7
	Malta	Tunisia	12

[a] Samples investigated (1991–1992) (1992–1993) harvesting periods. [b] Number of samples (total 124).

LC with a quaternary mobile-phase mixture. Precision and accuracy have been determined, and the amount of hesperidin and narirutin determined was investigated at different pH values of the juice. Multivariate statistical analyses were applied to 124 juice samples, for citrus juice classification, using FG determination. Such methods were successfully applied to citrus juice differentiation (Rouseff, 1988a).

Flavanone Glycosides of Citrus

*J. Agric. Food Chem.,* Vol. 42, No. 1, 1994 **71**
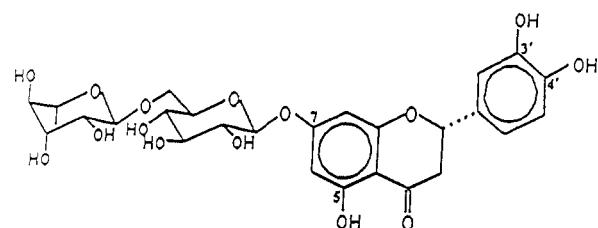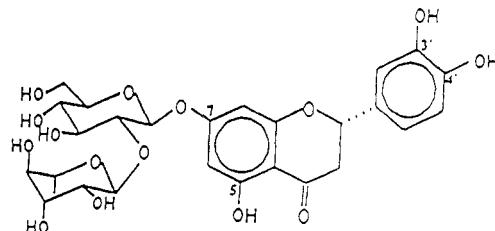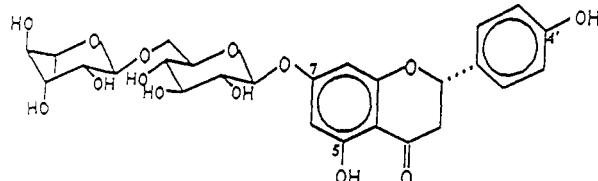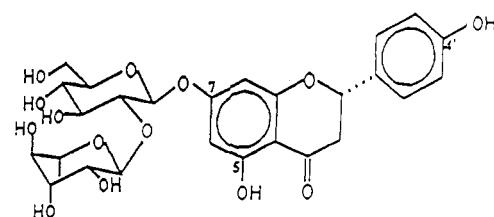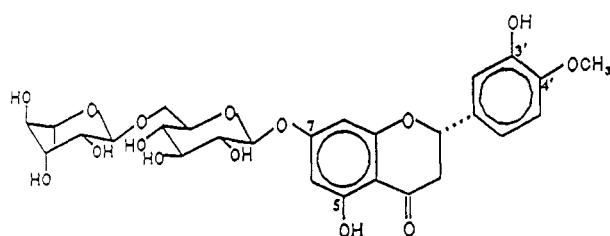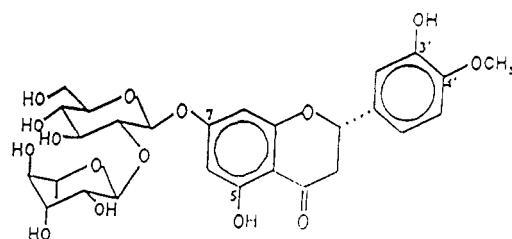
Figure 1. Flavanone glycosides investigated.

Principal component analysis (PCA) and factor discriminant analysis (FDA) of data obtained using liquid chromatography of FG from juices were applied to the four common species we have investigated (oranges, grapefruits, lemons, limes). The differentiation of subpopulations of these species (for lemon and lime; for grapefruits, white, pink, red, and green; and for sweet oranges, Valencia, navel, blood, Thomson, and Malta) was attempted.

MATERIALS AND METHODS

Standards. The six FG used as standards (Extrasynthese, France) were of analytical grade. Hesperidin was diluted in dimethylformamide (DMF)–water (2:1 v/v) to give a 200 mg L^{-1} concentration. All other reagents were of analytical grade and diluted in the mobile phase. Working standard solutions were prepared weekly by dilution with the mobile phase. The final concentrations were 20 mg L^{-1} for hesperidin and naringin and 10 mg L^{-1} for the other FG.

Materials. The different FG were studied on three commercial pure orange juices. Samples of other citrus fruits were purchased at a local market. The numbers, origin, and varieties of analyzed samples are given in Table 1.

Preparation of Samples. The citrus fruits were hand squeezed and juices filtered through a sieve (1.25 mm, Prolabo, France). The sample juices (5 mL) were diluted in DMF (10 mL) and in an ammonium oxalate solution (10 mL at 0.05 mol L^{-1}) and then placed on a steam bath for 10 min at 90 °C. After cooling, the solutions were adjusted to 50 mL in a volumetric
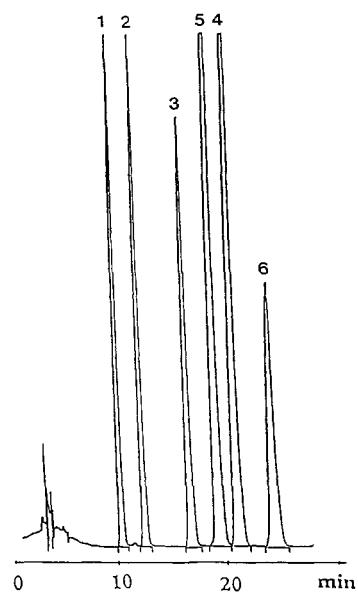

Figure 2. Separation of standard FG: column, 250 × 4.6 mm i.d.; stationary phase, RP18 Alltima; amount injected, 20 μL of a solution of 10 mg L^{-1} for compounds 1, 2, 3, and 6 and 20 mg L^{-1} for compounds 4 and 5; mobile phase, water–acetonitrile–THF–glacial acetic acid (80:16:3:1 v/v/v/v); inlet pressure, 19 MPa; temperature, ambient; flow rate, 1.5 mL min^{-1}; UV detection, 280 nm. For compound identification, see Table 2 and Figure 1.
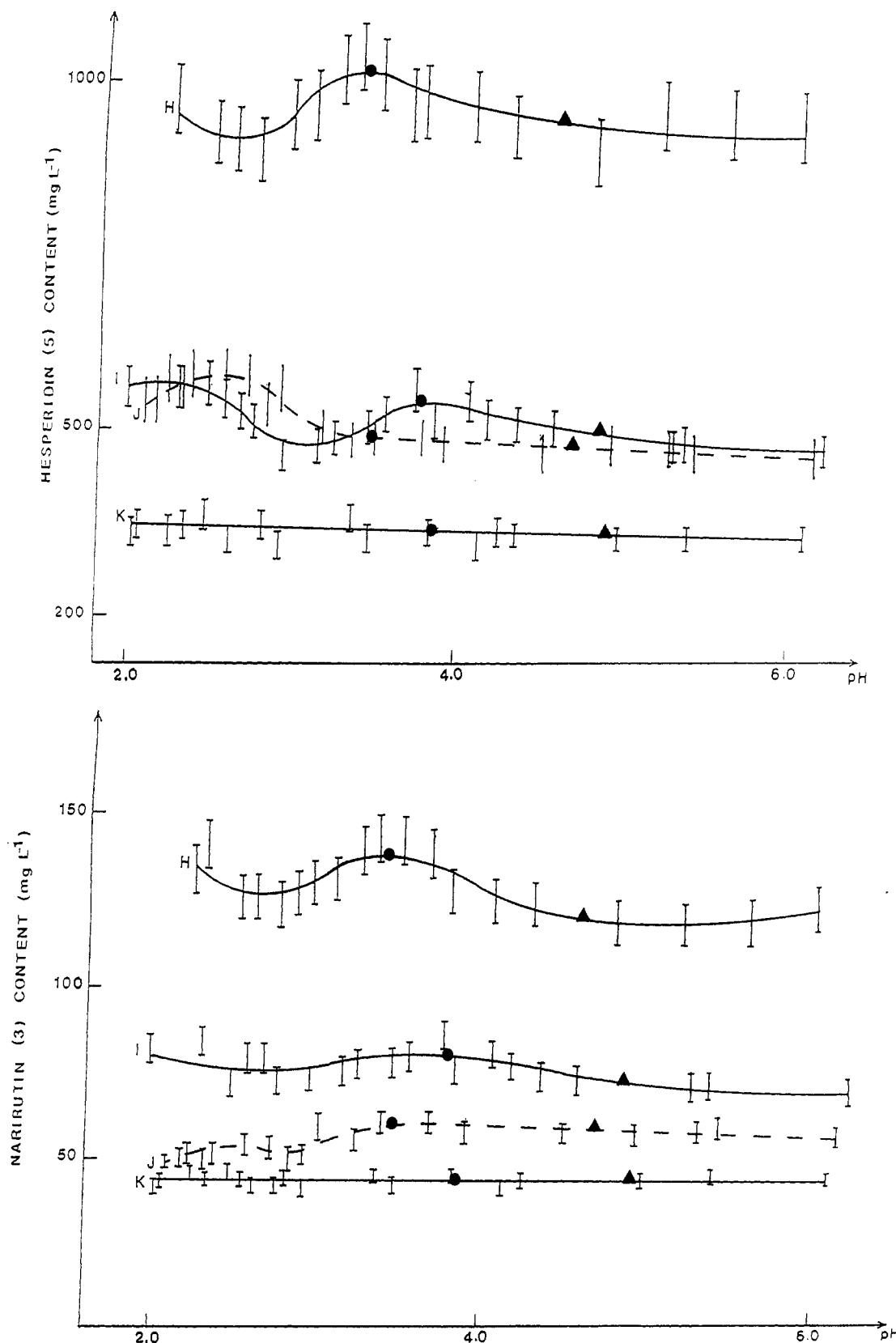
Figure 3. Flavanone glycoside extraction from pure commercial orange juices at various pH: (top) hesperidin 5 and (bottom) narirutin 3; (H) Spain; (I) Israel; (J) Morocco; (K) Florida; (●) pH value of initial pure commercial orange juice; (▲) pH value of orange juice after ammonium oxalate addition (see Materials and Methods).

flask. All solutions were centrifuged at high speed (2500g) for 10 min. The clarified juice solutions were filtered through Acrodisc filters (5 μm (acrylic polymers) and 0.45 μm (nylon), Gelman Sciences, France) and then injected in a 20-μL sample loop for LC analysis.

For pH range investigations on hesperidin and narirutin,

determinations were carried out using pure juice (to which aqueous saturated citric acid solution or NaOH at 3% w/v was previously added to obtain various pH values) before DMF addition, without ammonium oxalate addition.

The recovery was investigated using citrus juice samples having a high content in one FG: recovery of eriocitin in lemon (*Citrus*

Flavanone Glycosides of Citrus

J. Agric. Food Chem., Vol. 42, No. 1, 1994 **73**

Table 2. Flavanone Glycoside Composition in Citrus Fruits

compd[a]	trivial name	K'^b	R_f	sample[d]	mean[e] (mg L^{-1})	SD[f] (mg L^{-1})	CV[g] (%)	recovery[h] (%)
1 (ERI)	eriocitrin	0.445	1.159	ll	64	0.5	0.7	95
				li	75	3.9	5.2	95
2 (NER)	neoeriocitrin	0.632	1.273	SO	184	5.9	3.2	96
				SO	321	7.5	2.3	102
3 (NAT)	narirutin	0.846	1.100	OJ	51	1.2	2.4	102
				OJ	37	1.0	2.7	99
				OJ	128	2.8	2.2	102
4 (NAR)	naringin	1.090	1.139	G	205	2.5	1.2	100
				G	206	4.2	2.0	97
5 (HES)	hesperidin	1.000	1.084	OJ	293	9.5	3.2	105
				OJ	437	10.3	2.4	97
				OJ	915	9.7	1.1	98
6 (NEH)	neohesperidin	1.330	1.173	G	4.3	0.20	4.7	103
				G	11.7	0.39	3.3	100

[a] See Figure 1 for structure formulas. [b] Relative to hesperidin 5. [c] Response facto × 10[5]. [d] 11, lemon; li lime; SO, sour orange; OJ, orange juice, G, grapefruit. [e] Means of six determinations. [f] Standard deviation. [g] Coefficient of variation. [h] Recovery of flavanone glycoside added to citrus juice prior to extraction.
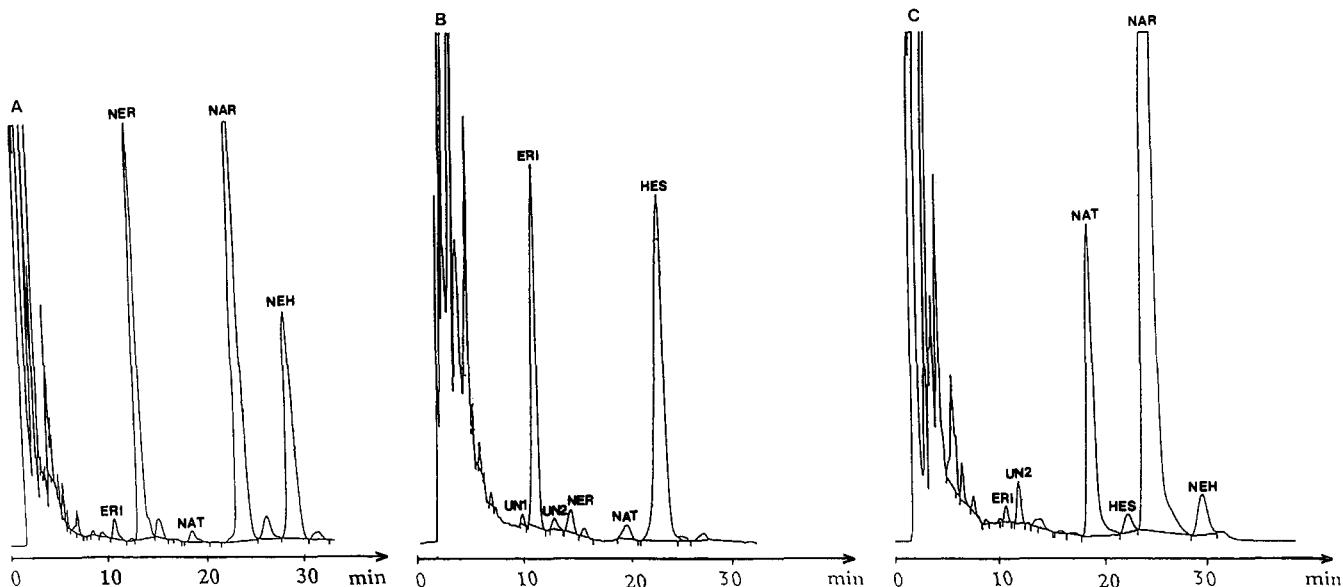

Figure 4. Chromatograms of citrus juice flavanone glycosides: (A) sour orange; (B) lemon; (C) white grapefruit. For chromatographic conditions see Figure 2. For peak identification see Table 3.

limon) primofiori and lime (*Citrus aurantifolia*); recovery of neoeriocitrin in sour orange (*Citrus aurantium*); recovery of narirutin and hesperidin in sweet orange (*Citrus sinensis*); and recovery of naringin and neohesperidin in grapefruit (*Citrus paradisi*). FG standards were added (about 20% more than determined content) to citrus juices, and the determinations in these new conditions were compared to calculated values.

Liquid Chromatography. Solvents and water were of HPLC grade. Separations were performed on a stainless steel column (250 × 4.6 mm i.d.) packed with RP-18 UHS, 5 µm (Alltech, France), equipped with a precolumn (30 × 4.6 mm i.d.) filled with the same stationary phase. The mobile phase was water–acetonitrile–tetrahydrofuran–glacial acetic acid (80:16:3:1 v/v/v/v). A Shimadzu LC 10 AS HPLC pump was used for analyses. Samples were introduced onto the column *via* a Rheodyne Model 7010 injector fitted with a 20-µL sample loop. A Shimadzu SPD 6 AV variable-wavelength UV–visible detector was set at 280 nm, and chromatographic data was obtained using a Shimadzu CR 5A integrator. The column was at ambient temperature, the inlet pressure was 19 MPa, and the flow rate was fixed at 1.5 mL min^{-1}. The FG contained in citrus juices were identified by comparison of their retention times with those of standards. For each sample solution, FG concentrations were determined using response factors obtained with the single-point external calibration method.

Table 3. Determination of Flavanone Glycosides in Citrus Groups

compd[a]	lemon + lime[c] mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	grapefruit[d] mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	sweet orange[e] mean (mg L^{-1})	SD (mg L^{-1})	CV (%)
1 (ER1)	70.9	23.7	33.4	tr[h]			3.0	2.23	74.3
2 (NER)	2.4	2.23	92.9	tr			nd		
3 (NAT)	5.1	3.10	60.8	96.7	64.4	66.6	56.8	26.9	47.4
4 (NAR)	nd[f]			246.8	133.7	54.4	nd		
5 (HES)	140.3	56.4	40.2	4.0	3.93	98.3	320.8	85.6	26.7
6 (NEH)	nd			5.2	4.38	84.4	nd		
7[b] (UN1)	0.4	0.33	82.5	tr			1.3	0.59	45.4
8[b] (UN2)	0.7	0.50	71.4	1.0	0.97	97.0	1.5	1.31	87.3
9[b] (UN3)	nd			nd			tr		

[a] See Table 2 and Figure 1 for name and structural formulas, concentration in mg L^{-1}. [b] Unknown compounds: relative percentage of total peak areas—see Figures 4 and 5. [c] Mean of 31 samples. [d] Mean of 42 samples. [e] Mean of 51 samples. [f] nd, not detected. [h] tr, traces: <0.1 mg L^{-1} for compounds 1–6; <0.01% for unknowns.

Statistical Analysis. Principal component analysis (PCA) has been performed by using the data set transformed into centered and reduced variables (standardized PCA). The data sets were first composed by all citrus samples (124) and all
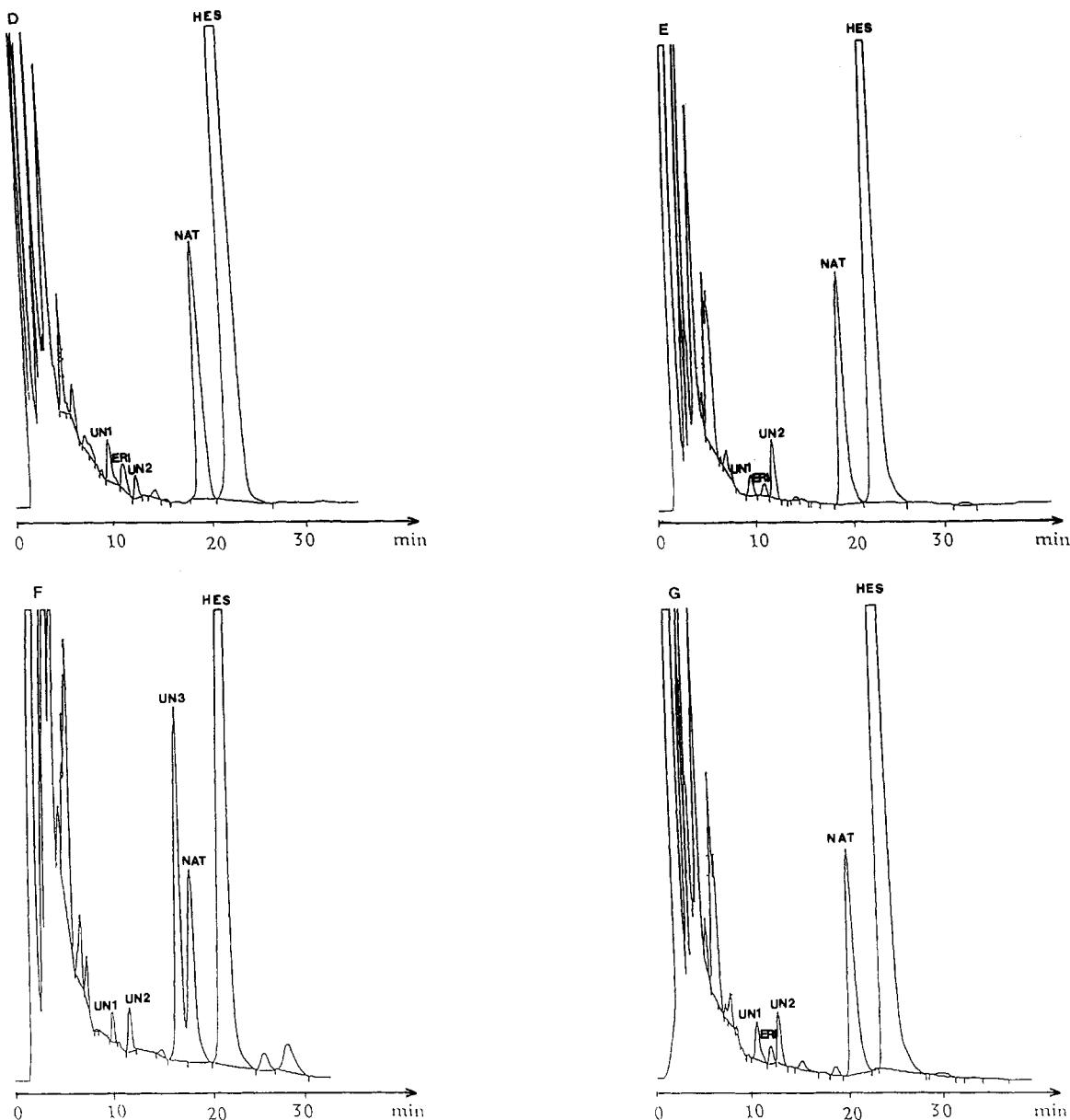
Figure 5. Chromatograms of sweet orange juice flavanone glycosides: (D) Valencia orange; (E) navel orange; (F) blood orange; (G) Malta orange. For chromatographic conditions see Figure 2. For peak identification see Table 6.

variables [eriocitrin, neoeriocitrin, narirutin, naringin, hesperidin, neohesperidin, and three unknown components (UN1, UN2, UN3)]. In a second attempt, for variety and geographical differentiation, data sets were composed as follows: for lemon and lime, by 31 samples and all FG except unknown 3; for grapefruits, by 42 samples and all FG except unknown 3; and for sweet oranges, by 51 samples and all variables except neoeriocitrin. Factor discriminant analysis (FDA) has been performed to classify into two or four subpopulations for lemon and lime, three or four subpopulations for grapefruits, and four or five subpopulations for sweet oranges. Juice data were processed with STATITCF program version 4 (ITCF, France) on an AT 486 microcomputer.

RESULTS AND DISCUSSION

Liquid Chromatography. The six flavanone glycoside standards commonly encountered in citrus (Figure 1) were easily separated by LC using a quaternary mixture as shown in Figure 2. From a comparison of these results with those previously observed (Mouly et al., 1993), better peak resolution of naringin and hesperidin was observed using a lower binding ratio of C_{18} reversed phase.

pH Influence on Flavanone Glycoside Determination. The influence of pH on the amount of hesperidin and narirutin determined from commercial orange juice using LC was investigated at different pH values (from 2.0 to 6.2). Results obtained are shown in Figure 3. Two pH ranges were highly effective for narirutin (2.0–2.5 and 3.2–4.0) as was one for hesperidin (3.2–4.0). The FG contents were unchanged at the pH 4.5–6.2 range. When these contents were low (30–50 and 300–400 mg L^{-1}, respectively), the determination were unaffected by a pH change. As shown in Figure 3, the pH value of orange juice was variable from pH 3.5 to 3.8. Therefore, a buffer, ammonium oxalate, was added to the orange juice to place the juice in a pH range where the determinations of narirutin and hesperidin were constant (pH 4.5–5.0).

Flavanone Glycoside Determination and Recovery. This FG determination method was applied to 14 various citrus juice samples, and each determination was repeated sixfold. The mean, standard deviation (SD), coefficient of variation (CV), and recovery are shown in Table 2. The average of the relative standard deviation for repeated

Flavanone Glycosides of Citrus

J. Agric. Food Chem., Vol. 42, No. 1, 1994 **75**

analyses is about 2.6%. The recovery range is between 95 and 105% with a mean of 99%.

Multivariate Statistical Analyses. Pattern recognition methods (Dagnelie, 1975; Jurs, 1986) with their multivariate data analysis capabilities can solve many complex problems. A lot of these methods are effective to combat adulteration (Perfetti *et al.*, 1988; Page *et al.*, 1988; Widmer *et al.*, 1992).

The flavanone glycoside patterns of citrus species we have studied are sufficiently distinctive to permit the discrimination of many juices using principal component analysis (PCA) or other multivariate approaches. Chromatograms using LC with a quaternary solvent eluent system of citrus juices are given in Figure 4 for sour orange, lemon, and white grapefruit. In the case of sweet oranges (Figure 5), the relatively high amounts of three unknown compounds are characteristics of Valencia, navel, blood, Thomson, and Malta orange varieties. Our results for 124 samples are summarized in Table 3. The major FG in each citrus group were as follows: in lemon and lime, eriocitrin (47–94 mg L^{-1}) and hesperidin (84–196 mg L^{-1}); in sweet orange, narirutin (30–84 mg L^{-1}) and hesperidin (235–407 mg L^{-1}); in grapefruit, narirutin (33–161 mg L^{-1}) and naringin (113–481 mg L^{-1}).

Unknown component 1 has been detected in lemon and lime, green grapefruits and oranges, unknown component 2 in all categories except green grapefruits, and unknown component 3 in Malta oranges and in higher amount in blood oranges. An examination of the principal components of the eigenvectors generated by discriminant analysis could indicate the more effective variables in separating lemon, sweet orange, and grapefruit into the correct species classification (Figure 6). Sweet oranges, highly correlated with narirutin and the three unknown compounds, were differentiated from lemon and lime and grapefruits by axis 1 (73% of total variance). Axis 2 (27% of the total variance), which is highly positively loaded with eriocitrin and negatively with narirutin, naringin, and neohesperidin, differentiated lemon and lime from grapefruits (Figure 6). Since these species were well separated into various citrus juice families, subsequent multivariate anlayses were performed on each species.

The means, standard deviations, and coefficients of variation of FG determinations in 124 samples of lemon and lime, grapefruits, and oranges are given in Tables 4–6, respectively, taking into account their origins and varieties. Eriocitrin was present in large amount in lemon (78–88 mg L^{-1}) but only at 49–62 mg L^{-1} in lime. Neoeriocitrin was found in low amount in lemon and lime (3–4 mg L^{-1}) and green grapefruits (6 mg L^{-1}). Narirutin was present in every sample (3–179 mg L^{-1}); the lowest concentration was found in lime (1.7–7.4 mg L^{-1}). All categories contained hesperidin (1–380 mg L^{-1}); grapefruit was an exception with a low content in this FG (1–5 mg L^{-1}). Sweet oranges and lemons contained neither naringin nor neohesperidin; naringin was the major FG of grapefruits (160–330 mg L^{-1}). Grapefruits were characterized by higher amount in neohesperidin (4–8 mg L^{-1}). No significant differences were observed between varieties.

In a standardized principal component analysis six variables (ERI, NER, NAT, HES, UN1, and UN2) were used to classify the different origins of lemon and lime, eight variables (UN3 is unused) to classify the four varieties of grapefruit, and six variables (ERI, NAT, HES, UN1, UN2, and UN3) to classify the five varieties of sweet orange as shown in Tables 4–6. The correlation matrices show highly positive correlation between eriocitrin and narirutin (*r* = 0.73) and between eriocitrin and hesperidin (*r* = 0.71)
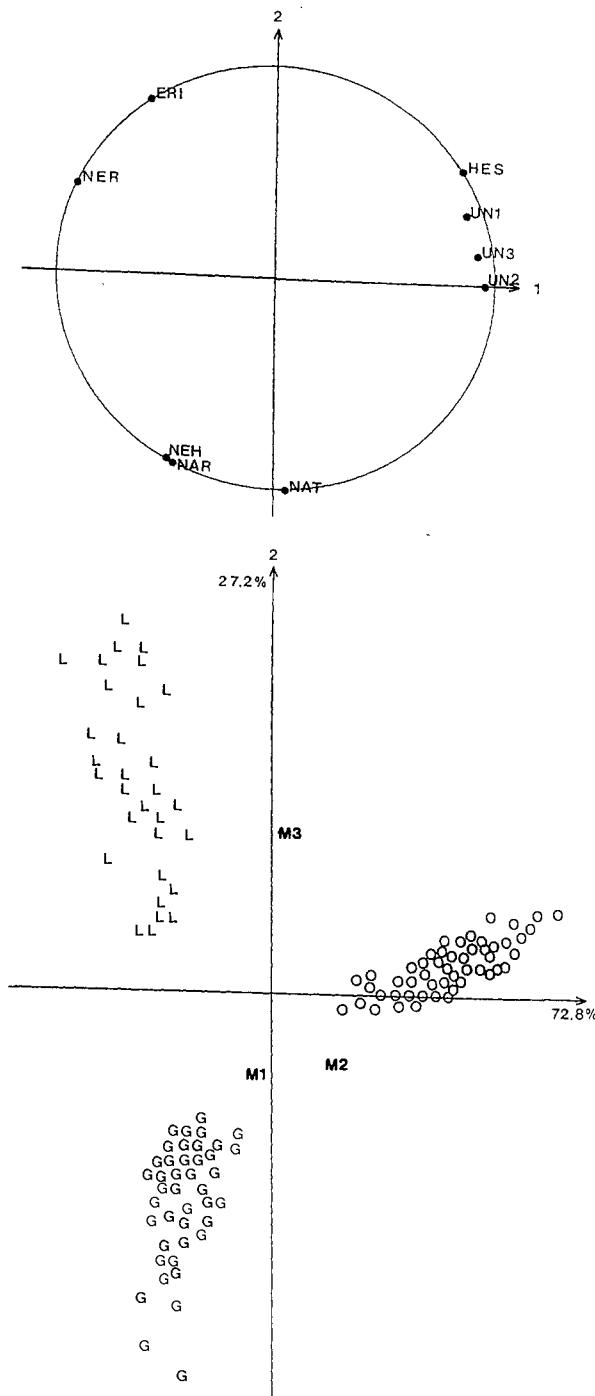

Figure 6. Citrus juice differentiation using FDA of flavanone glycoside contents. (Top) Factor loading of variables on the two discriminant axes. For compound identification see Figure 1 and Table 3. (Bottom) Two-dimensional plot of lemon and lime (L), grapefruit (G), and sweet orange (O) samples investigated. M1, mixture of pink grapefruit and Malta orange; M2, mixture of white grapefruit and Valencia orange; M3, mixture of lemon and Valencia orange.

for the lemon and lime group, highly positive correlation coefficient between eriocitrin and neoeriocitrin (*r* = 0.85) and between hesperidin and neohesperidin (*r* = 0.72) for grapefruit, and a negative correlation coefficient between eriocitrin and unknown 3 (*r* = 0.48) for the orange group. It can be observed that the three first principal components (PC) represent 84.5% of the cumulated variance for the lemon and lime group, 78% for the grapefruit group, and 78% for the orange group. The representation of samples on the two first PC shows a beginning of differentiation

Table 4. Determination of Flavanone Glycoside in *C. limonia* and *C. aurantifolia*

| | lemon | | | | | | lime | | | | | |
| | Spain[c] | | | France[d] | | | Brazil[e] | | | Mexico[f] | | |
compd[a]	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)
1 (ERI)	87.7	18.4	21.0	78.1	17.2	22.0	48.5	15.0	31.0	62.4	20.0	32.0
2 (NER)	4.1	1.72	42.0	nd[g]			3.3	0.37	11.2	3.9	1.79	45.9
3 (NAT)	6.8	3.33	49.0	5.1	1.94	38.0	2.9	1.22	42.1	5.2	2.18	55.9
5 (HES)	154.4	54.0	35.0	116.3	46.5	40.0	108.8	32.6	30	167.8	65.4	39.0
7[b] (UN1)	0.9	0.34	37.8	nd			tr[h]			tr		
8[b] (UN2)	0.9	0.24	26.7	1.3	0.44	33.9	0.8	0.26	32.5	0.9	0.13	14.4

[a] See Table 2 and Figure 1 for name and structural formulas, concentration in mg L^{-1}. [b] Unknown compounds: relative percentage of total peak areas. See Figure 4. [c] Mean of 13 samples. [d] Mean of 4 samples. [e] Mean of 8 samples. [f] Mean of 6 samples. [g] nd, not detected. [h] tr, traces: <0.1 mg L^{-1} for compounds 1–6; <0.01% for unknowns.

Table 5. Determination of Flavanone Glycosides in *C. paradisi*

| | white[c] | | | pink[d] | | | red[e] | | | green[f] | | |
compd[a]	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg mL^{-1})	CV (%)
1 (ERI)	3.1	1.80	58.1	tr			tr			6.2	3.41	55.0
2 (NER)	nd[g]			nd			nd			5.9	2.48	42.0
3 (NAT)	105.7	70.82	67.0	56.1	20.20	36.0	76.0	28.88	38.0	178.6	55.37	31.0
4 (NAR)	331.3	185.5	56.0	159.1	50.91	32.0	275.4	115.67	42.0	251.6	55.35	22.0
5 (HES)	5.3	4.02	75.9	4.6	3.04	66.1	5.2	4.63	89.0	1.4	0.94	67.1
6 (NEH)	7.8	6.47	83.0	4.7	2.26	48.1	6.6	3.10	47.0	3.9	1.33	34.1
7[b] (UN1)	tr[h]			nd			nd			0.3	0.11	36.7
8[b] (UN2)	2.1	0.88	41.9	0.8	0.34		0.6	0.38	64.0	tr		

[a] See Table 2 and Figure 1 for name and structural formulas, concentration in mg L^{-1}. [b] Unknown compounds: relative percentage of total peak areas. See Figure 4. [c] Mean of 11 samples. [d] Mena of 14 samples. [e] Mean of 9 samples. [f] Mean of 8 samples. [g] nd, not detected. [h] tr, traces: <0.1 mg L^{-1} for compounds 1–6; <0.01% for unknowns.

Table 6. Determination of Flavanone Glycosides in *C. sinensis*

| | Valencia[c] | | | navel[d] | | | blood[c] | | | Thomson[f] | | | Malta[g] | | |
compd[a]	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)	mean (mg L^{-1})	SD (mg L^{-1})	CV (%)
1 (ERI)	3.1	1.21	39.0	3.6	0.76	21.1	nd[h]			5.0	2.95	59.0	3.1	2.02	65.2
3 (NAT)	36.9	8.12	22.0	85.1	12.8	15.0	43.3	17.81	41.0	80.3	31.3	39.0	39.7	8.3	21.0
5 (HES)	230.0	48.3	21.0	379.3	79.7	21.0	363.0	65.3	18.0	309.6	49.5	16.0	304.3	76.1	25.0
7[b] (UN1)	2.0	0.44	22.0	1.4	0.24	17.1	1.3	0.30	23.1	1.4	0.31	22.1	0.7	0.32	45.7
8[b] (UN2)	0.9	0.22	24.4	2.8	1.54	55.0	1.8	0.36	20.0	1.6	0.72	45.0	2.31	0.92	40.0
9[b] (UN3)	nd			nd			11.0	8.25	75.0	nd			0.9	0.39	43.3

[a] See Table 2 and Figure 1 for name and structural formulas, concentration in mg L^{-1}. [b] Unknown compounds: relative percentage of total peak areas. See Figure 5. [c] Mean of 11 samples. [d] Mean of 12 samples. [e] Mean of 9 samples. [f] Mean of 7 samples. [g] Mean of 12 samples. [h] nd, not detected.

between various subgroups. Therefore, factorial discriminant analyses (FDA) using the different data sets were applied for the characterization of discriminant FG. Each *Citrus* species was classified by FDA as follows: 91% for grapefruits if we consider three subgroups (white, red plus pink, green), 94% for oranges if we consider four subgroups (Valencia, navel plus Thomson, blood, and malta), and 100% for lemon and lime. An attempt of pink and red grapefruit differentiation leads to 75% correct classification. Differentiation from French and Spanish lemon and Brazilian and Mexican lime leads to 84% correct classification. Differentiation of navel and Thomson oranges leads to 86% correct attribution.

The graphical representation of variables and samples for lemon and lime are given in Figure 7. The discriminant power of axis 1, which represents 68.2% of the total variance and is loaded with narirutin and eriocitrin, gives the separation of lemons from limes. Axis 2, which is loaded with hesperidin, represents 24.2% of the total variance and gives both separations of Brazilian lime, in the negative part of this axis, from Mexican lime in the

positive part. A beginning of Spanish and French lemon differentiation [Figure 7 (bottom)] was obtained on this axis. The third axis (15% of total variance) was not able to enhance this differentiation.

Graphical representations of variables and grapefruit samples on axes 1 and 2 are given in Figure 8. The discriminant power of axis 1, which represents 70.6% of the total variance, gives the separation of green grapefruits in the negative part of this axis, which is highly loaded with eriocitrin and neoeriocitrin. Unknown component 2 mainly contributes in the discrimination of white grapefruits. As shown in Figure 8 (bottom), the pink and red varieties have the same behavior. The third FDA axis was not able to differentiate these two varieties.

The graphical representation of variables and samples of sweet oranges on axes 1 and 2 and 1 and 3 are given in Figure 9. The high content in narirutin contributes to separate navel and Thomson oranges from other varieties (80–85 vs 37–43 mg L^{-1}). Axis 2, highly loaded with hesperidin, is effective in separating blood from Valencia

Flavanone Glycosides of Citrus

J. Agric. Food Chem., Vol. 42, No. 1, 1994 **77**
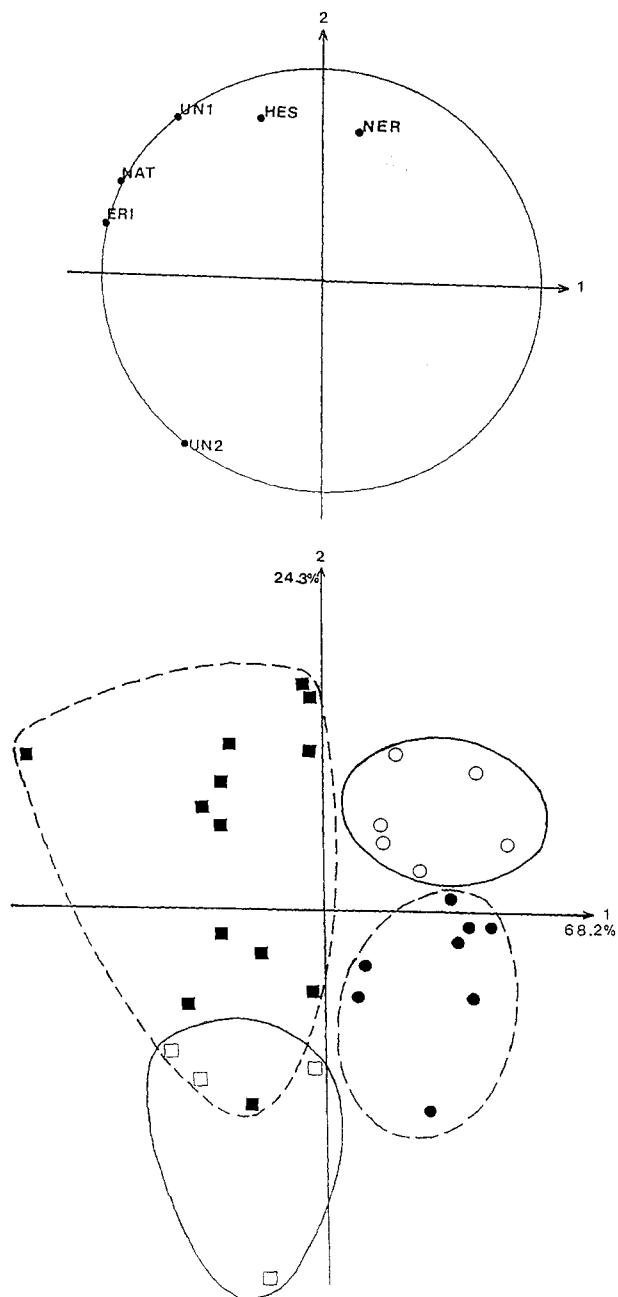
Figure 7. Geographical and variety juice differentiation using of lemon and lime FDA of flavanone glycoside contents. (Top) Projection of factor loading variables on the two main discriminant axes. For compound identification see Figure 1 and Table 4. (Bottom) Two-dimensional plot of the samples investigated: (■) Spanish lemons; (□) French lemons; (●) Brazilian lime; (O) Mexican lime.

oranges (360 vs 230 mg L⁻¹). Although some overlapping occurs, additional examination of axis 3 (15.2% of the total variance), highly loaded with unknown component 1 (−0.96), gives better differentiation of Malta from Valencia and blood oranges [Figure 9 (bottom right)]. These plots give a qualitative picture of the usefulness of FG content in distinguishing the categories investigated.

The flavanone glycoside profile including three unknown compounds and FG patterns of these citrus species are distinctive to permit the determination of the probable composition of a simple mixture of juices [Figure 6 (bottom)].

Conclusion. Flavanone glycoside compositions, easily determined by isocratic LC, are useful in the differentiation
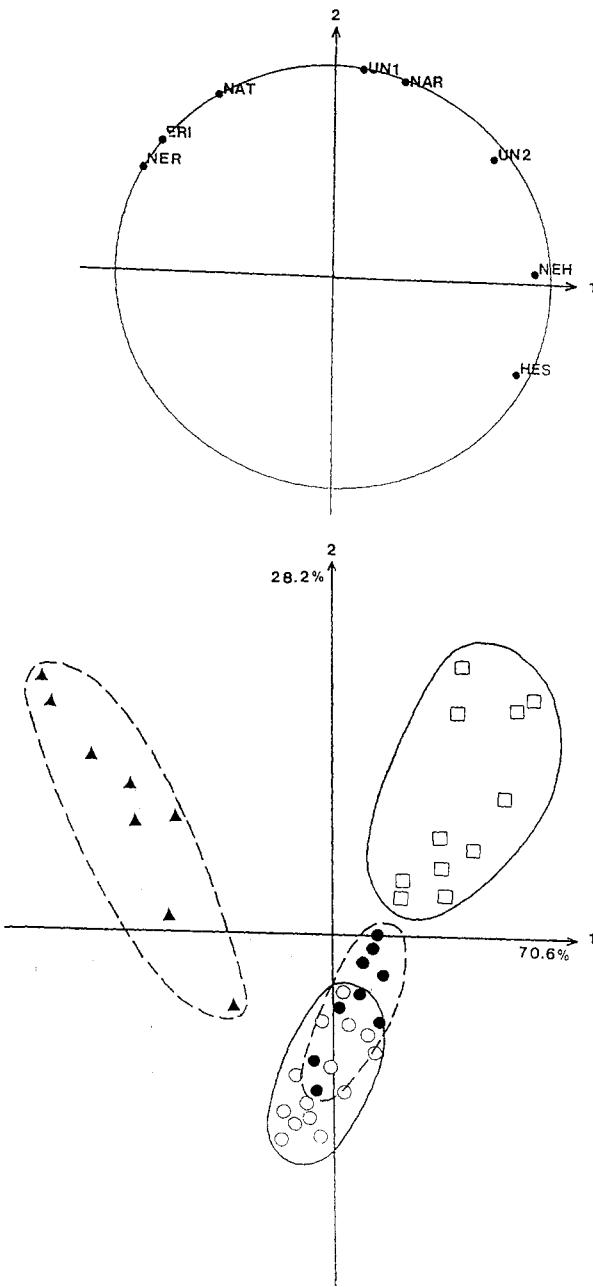
Figure 8. Grapefruit juice differentiation using FDA of flavanone glycoside contents. (Top) Projection of factor loading variables on the two main discriminant axes. For compound identification see Figure 1 and Table 5. (Bottom) Two-dimensional plot of the samples investigated: (□) white; (O) pink; (●) red; (▲) green.

of citrus species and permit the determination of the probable composition of simple mixtures of citrus juices. Using pattern recognition techniques, sweet oranges, grapefruits, and lemons were easily distinguished. Some varieties from the same species with similar FG profile cannot be separated: pink from red grapefruits, navel from Thomson oranges. However, the differentiation of lemon and lime, Valencia, blood, and Malta oranges, or white and green grapefruits was achieved. FG determination associated with multivariate statistical analysis seems interesting for citrus juice inspections.

78   *J. Agric. Food Chem.*, Vol. 42, No. 1, 1994

Mouly et al.
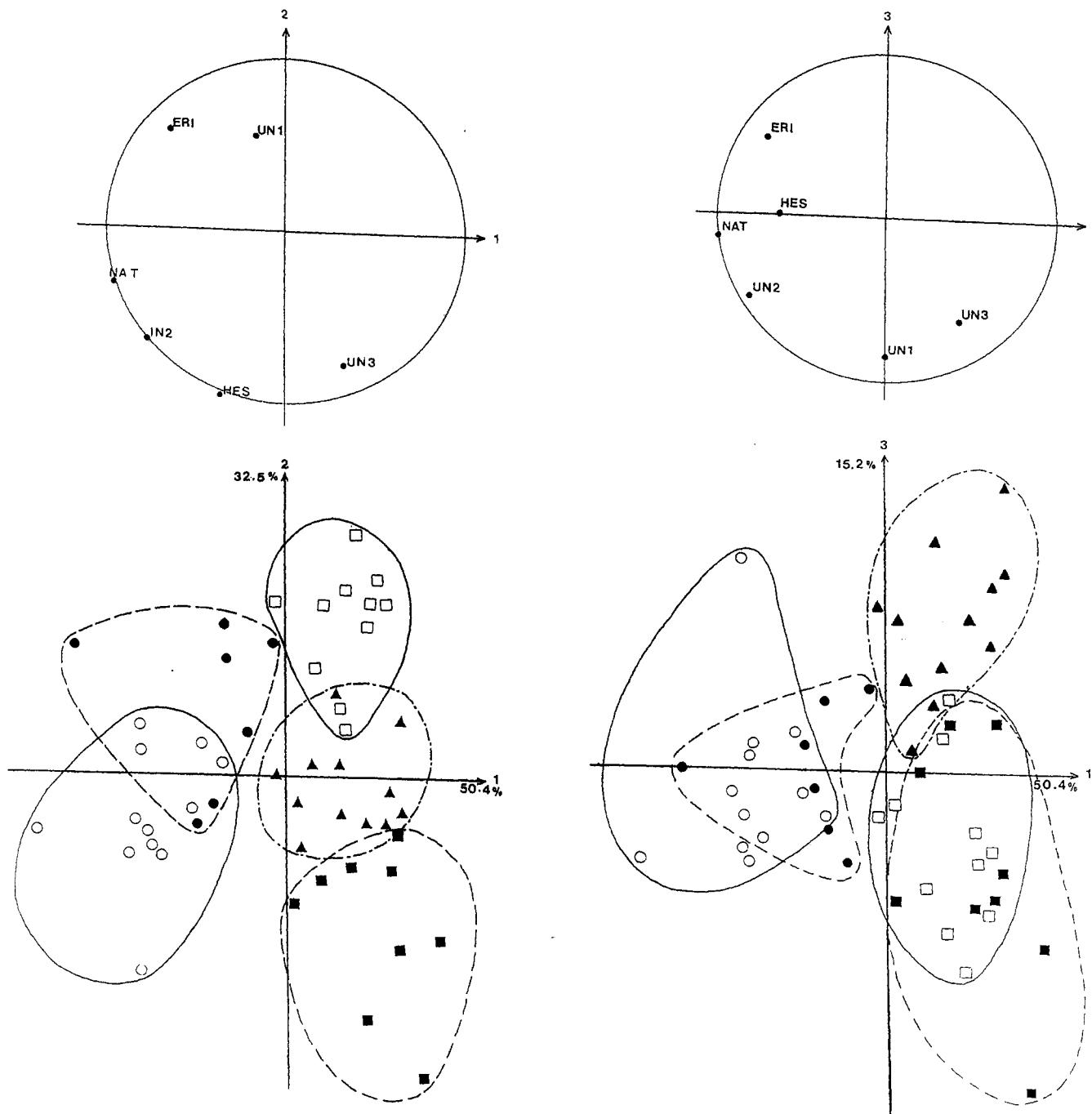
Figure 9. Sweet orange juice differentiation using FDA of flavanone glycoside contents. For compound identification see Figure 1 and Table 6. (Top) Projection of factor loading variables on discriminant axes 1 and 2 (left) and 1 and 3 (right). (Bottom) Two-dimensional plot of the samples investigated on discriminant axes 1 and 2 (left) and 1 and 3 (right). (□) Valencia; (O) navel; (■) blood; (●) Thomson; (▲) Malta.

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Flavanone Glycosides of Citrus

J. Agric. Food Chem., Vol. 42, No. 1, 1994 **79**

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