Blood Orange Juice Authentication Using Cinnamic Acid Derivatives. Variety Differentiations Associated with Flavanone Glycoside Content

Pierre P. Mouly,† Emile M. Gaydou,*,‡ Robert Faure,‡ and Jacques M. Estienne§

Centre de Recherche et de Valorisation des Produits de Consommation, Parc Club des Aygalades, 35 Bd du Capitaine Gèze, F-13333 Marseille Cedex 14, France, Laboratoire de Phytochimie de Marseille and URA CNRS 1411, Faculté des Sciences et Techniques de Saint-Jérôme, Avenue Escadrille Normandie Niemen, F-13397 Marseille Cedex 20, France, and Institut de Chimie Analytique et du Contrôle de la Qualité, Université de Droit d'Economie et des Sciences d'Aix-Marseille, Avenue R. Schuman, 13380 Aix enProvence, France

A phenyl propanoid glycoside, cinnamoyl-β-D-glucopyranoside (CIN), characteristic of blood oranges, was isolated by column and thin layer chromatographies. The structure of this compound was established using ^{1}H and ^{13}C NMR spectral data and by analysis of its components upon hydrolysis. Flavanone glycosides (FGs) [narirutin (NAT), hesperidin (HES), and didymin (DID)] and cinnamic derivatives [CIN and *trans*-cinnamic acid (TCA)] of four blood orange varieties (47 samples) were analyzed by HPLC. Differences in the polyphenol profiles clearly showed variety differentiation.

Keywords: *Fruit juice; Citrus sinensis; blood orange varieties; cinnamoyl-β-D-glucopyranoside; flavanone glycoside; NMR; liquid chromatography*

INTRODUCTION

Blood oranges are *Citrus sinensis* (Rutaceae family) varieties originating from Malta or Sicilia islands and have been cultivated, for many centuries, in Mediterranean areas, especially in Italy, Spain, Morocco, and Tunisia and more recently in California and Florida (Saunt, 1990). The peel and pulp colorations are explained by the presence of red anthocyanins (Maccarone et al., 1985; Lee et al., 1990).

The genus *Citrus* is characterized by substantial accumulation of flavanone glycosides (FGs). Four aglycons are generally involved, all of them being 5,7-di-OH-substituted: naringenin, eriodictyol, and their 4′-methylated derivatives isosakuranetin and hesperetin (Macheix et al., 1990; Harborne, 1992). Flavanones in citrus fruits are of chemotaxonomic interest (Kamiya et al., 1979) and are used for citrus juice authentication. Each species of *Citrus* is characterized by a particular FG pattern obtained using liquid chromatography (LC) (Fisher, 1976) which is used for species differentiation (Rouseff et al., 1987, 1988). Flavanone profile determination by LC, for quality control of citrus juices, is now widespread to detect the adulteration by mixture of *Citrus* species or varieties (Perfetti et al., 1988; Reminiac et al., 1989; Viguera et al., 1993; Mouly et al., 1995). During the course of FG separations and identifications for *Citrus* juice quality specifications (Mouly et al., 1993, 1994), we detected, in blood orange juices, the presence of an unidentified compound, in relatively high amounts. In looking for the chemical nature of this compound, we found a new phenylpropanoid glycoside in blood orange which is described in this paper. On the other hand, we have studied the composition of FGs of four blood orange varieties for their differentiation.

Table 1. Gradient Profile Used in Liquid Chromatography Separation of Flavanone Glycosides of Blood Orange Juices

	time (min)			
	0	25	30	35[b]
% of B[a]	83	60	60	83
nature of gradient	concave	isocratic	linear	

[a] Solvent B: water/acetic acid, 96/4 v/v. Solvent A: acetonitrile. [b] Equilibrated time = 10 min.

MATERIALS AND METHODS

Materials. Forty-seven samples of four varieties of blood oranges (Washington sanguine, WHS; Malta, ML; Sanguinelli, SN; Moro, MO) were purchased on a local market of Marseilles during the period from January to April 1996.

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 C18 Alltima, 5 μm (Alltech, France), equipped with a precolumn (7.5 × 4.6 mm i.d.) filled with the same stationary phase. The gradient profile and the mobile phase composition are given in Table 1. A Waters 600 controller pump was used for analyses. Samples were introduced onto the column via an automatic injector (Waters 717) equipped with a sample loop (20 μL). A Waters 996 diode array detector was set at 280 nm, and chromatographic data were handled with a Millenium driver station. The column was at ambient temperature, the inlet pressure was 15 MPa, and the flow rate was fixed at 1.0 mL min^{-1}.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer in CD_3OD solutions (TMS as standard). ^{13}C resonance multiplicities were established via the acquisition of DEPT spectra (Doddrell et al., 1982).

Standards. The three flavanone glycosides (FG), hesperidin (HES), narirutin (NAT), and didymin (DID). and *trans*-cinnamic acid (TCA) were of analytical grade and used as standards (Extrasynthèse, France). HES was diluted in dimethylformamide (DMF)−water (2/1 v/v) to give a 200 mg L^{-1} solution. The other reagents were diluted in the mobile

* To whom correspondence should be addressed.
† Centre de Recherche et de Valorisation des Produits de Consommation.
‡ Faculté des Sciences et Techniques de Saint-Jérôme.
§ Université de Droit d'Economie et des Sciences d'Aix-Marseille.

S0021-8561(96)00509-2 CCC: $14.00

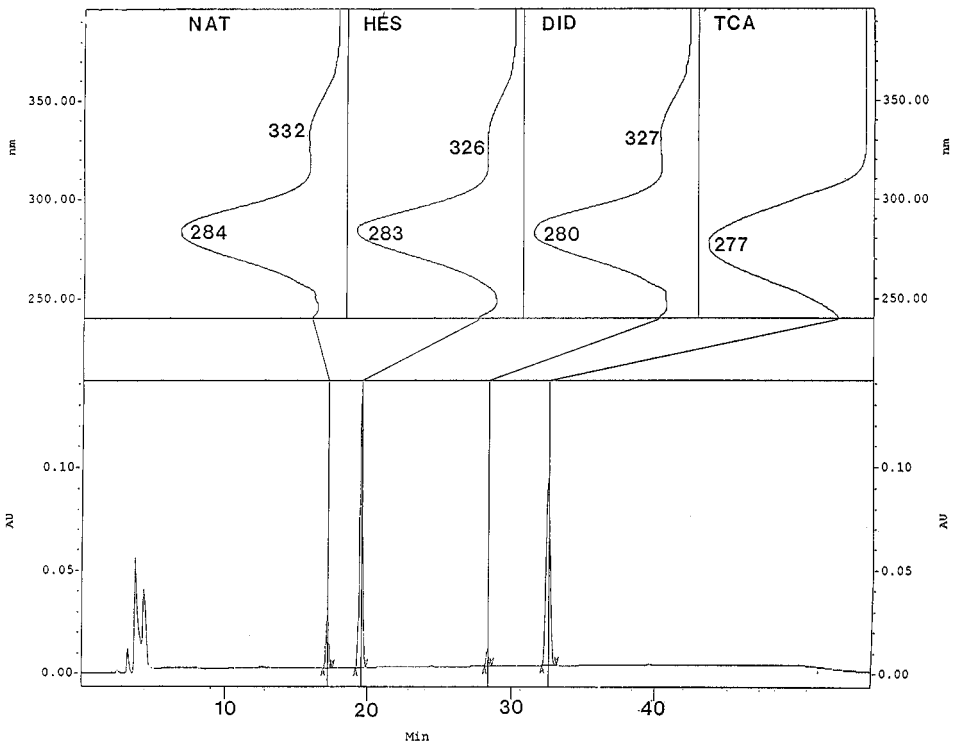


Figure 1. Separation and identification of flavanone glycoside standards and *trans*-cinnamic acid: column, 250 × 4.6 mm i.d.; stationary phase, C18 Alltima; amount injected, 20 μL of a solution at 5 mg L^{-1} for didymin (DID), 10 mg L^{-1} for narirutin (NAT), 15 mg L^{-1} for *trans*-cinnamic acid (TCA), and 20 mg L^{-1} for hesperidin (HES); UV detection, 280 nm. For gradient elution and nature of solvents, see Table 1; for compound identification, see Table 3.

phase to give 100 mg L^{-1} for NAT and DID and 50 mg L^{-1} for TCA. Working standard solutions were prepared weekly by dilution with the mobile phase. The final concentration were 20 mg L^{-1} for HES, 10 mg L^{-1} for NAT and DID, and 5 mg L^{-1} for TCA.

Preparation of Blood Orange Juice Samples. The blood orange fruits were hand-squeezed and juices filtered through a sieve (1.25 mm, Prolabo, France). There were two different preparations of samples because the concentration of TCA was smaller than that for FG and CIN. In the cases of FG and CIN determinations, the blood orange juices (5 mL) were diluted in DMF (10 mL) and in a ammonium oxalate solution (10 mL at 0.05 mol L^{-1}) to fix the pH (Mouly et al., 1994) 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 flask. All solutions were centrifuged at high speed (2500*g*) 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 into a 20 μL sample loop for LC analysis. In the case of TCA determination, the blood orange juices (10 mL) were centrifuged at high speed (2500*g*) for 10 min and concentrated on a reversed phase cartridge. The reversed phase cartridge (C18, Sep-Pack Plus, Waters) was conditioned with successively 5 mL of acetonitrile (1 min) and 2 × 5 mL of water (2-fold, 1 min). Clarified sample (10 mL) was injected through the cartridge during 2 min, washed with 5 mL of water during 1 min, then dried under an air stream. The compounds retained on the cartridge were removed with 5 mL of acetonitrile during 1 min. The solvent was evaporated under reduced pressure at 40 °C. The resulting dried matter was dissolved in 1 mL of the mobile phase and then injected into a 20 μL sample loop for HPLC analysis.

The FG and the TCA concentrations were determined using response factor obtained with the single-point external calibration method. The final concentration of each FG and TCA is expressed in mg L^{-1} (Figure 1).

Isolation Procedure of Cinnamoyl-β-D-Glucopyranoside. Blood *Citrus sinensis* fruits, Moro variety (10 kg), were cut in half and hand squeezed. The juice (3.5 L) was filtered through a sieve (1.25 mm), clarified by centrifugation, and chromatographied by column chromatography on C18. Elution was achieved first using methanol, then with 80% aqueous acetonitrile. The compounds eluted were fractionated by a combination of preparative precoated RP-18 TLC glass plates from Machery Nagel. The following solvent systems were employed: water−acetonitrile−tetrahydrofuran (THF)−acetic acid (85/12/2/1% v/v/v/v) then water−acetonitrile−THF−acetic acid (81/14/2/3% v/v/v/v) to increase the separation of the compound from narirutin. The pure compound (14 mg), R_f 0.43 (UV at 254 nm, pale yellow absorbances), was scraped off, eluted from C18 phase with methanol, and submitted to NMR analyses and acid hydrolysis. Purity of the compound was controlled by LC, using a Shimadzu LC 10 AS HPLC pump [(k' = 0.632) relative to hesperidin]. For detail chromatographic conditions see Mouly et al. (1994).

Acid Hydrolysis of Cinnamoyl-β-D-Glucopyranoside (Figure 2). A solution of the compound in 4 M HCl (2 mL) was refluxed for 1 h. Then water was added and the mixture was extracted with chloroform. The aqueous layer was neutralized with sodium hydrogen carbonate. The presence of cinnamic acid was confirmed using LC and UV detector, by comparison with a standard (Figure 2). Glucose was characterized by enzymatic analysis (Enzymatic test of sucrose/D-glucose/D-fructose, Boehringer Mannheim).

RESULTS AND DISCUSSION

Identification of Cinnamoyl-β-D-Glucopyranoside in Blood Orange. In a previous paper (Mouly et al., 1994) we have found an unknown compound which characterized blood orange juices, called UN3. This compound, present in large amount in Moro variety has now been isolated and identified. Fractionation of blood orange juice, using column and thin layer chromatographies, led to the isolation of a pure compound. Acid hydrolysis of this compound yielded cinnamic acid and a sugar, which was found to be glucose. The remaining problem was to assign the position attachment of cinnamic acid on the glucose moiety. The ^{1}H NMR signals indicated that the isolated compound was con-

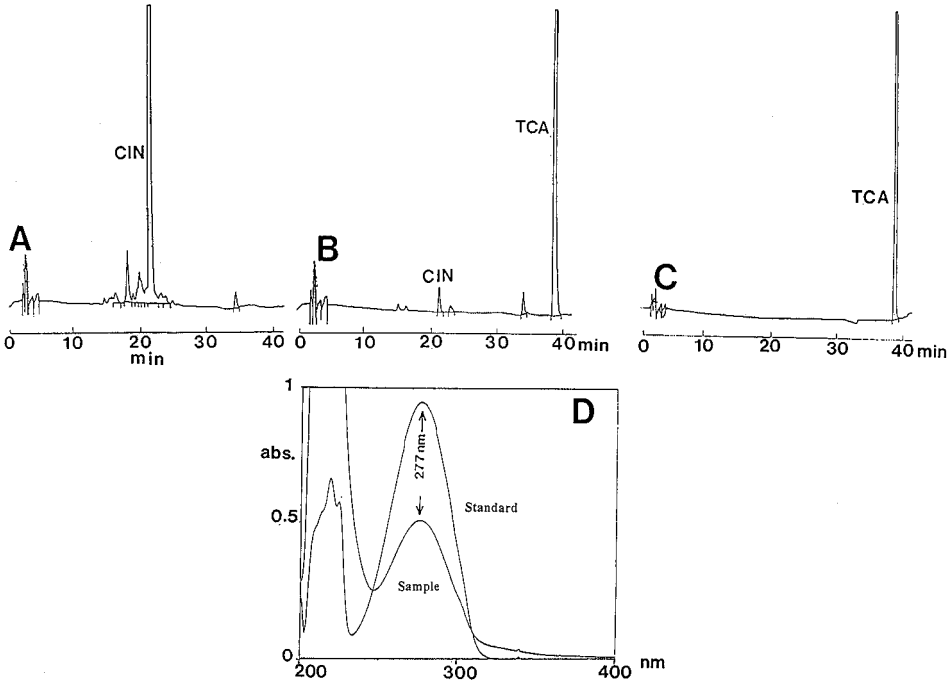
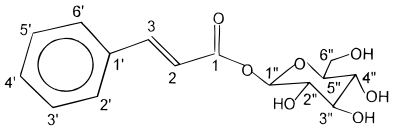


Figure 2. Liquid chromatography of isolated cinnamoyl-β-D-glucopyranoside (CIN) for blood orange juice before (A) and after (B) hydrolysis and of standard *trans*-cinnamic acid (TCA) (C). UV spectra comparison of cinnamoyl-β-D-glucopyranoside after hydrolysis and TCA (D).

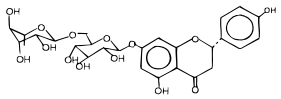
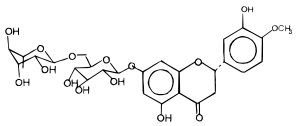
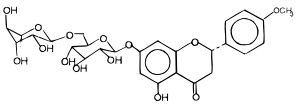
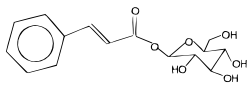
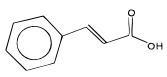
Table 2. ^{1}H and ^{13}C Chemical Shift of Cinnamoyl-β-D-glucopyranoside



δ ^{13}C[a]	group[b]	assignment	δ ^{1}H[a]
167.1	C	C-1	—
147.7	CH	C-3	7.80 (J = 16.1 Hz)
135.6	C	C-1′	—
131.8	CH	C-4′	7.40
130.1	CH	C-2′, C-6′	7.62
129.4	CH	C-3′, C-5′	7.40
118.3	CH	C-2	6.58 (J = 16.1 Hz)
96.0	CH	C-1″	5.59 (J = 7.7 Hz)
78.9	CH	C-3″	
78.0	CH	C-5″	
74.1	CH	C-2″	3.55−3.50
71.5	CH	C-4″	
62.4	CH_2	C-6″	3.85 (dd J = 12.1 and 1.9 Hz)
			3.68 (dd J = 12.1 and 4.8 Hz)

[a] In ppm with respect to TMS. [b] Determined from DEPT spectra.

Table 3. Names, Formula, and k' of Compounds Investigated

Compound	Trivial name	Formula	k'[a]
(**NAT**)	Narirutin		0.772
(**HES**)	Hesperidin		1.000
(**DID**)	Didymin		1.468
(**CIN**)	Cinnamoyl-β-D-glucose		0.810
(**TCA**)	*Trans*-cinnamic acid		1.588

[a] Relative to hesperidin.

sistent with the presence of cinnamic acid, with two multiplets centered at δ 7.62 (2H) and 7.40 (3H), corresponding to the aromatic protons and an AX system belonging to the *trans*-olefinic protons at δ 7.80 and 6.58 (each 1H, d, J = 16.1 Hz). The anomeric proton appeared at δ 5.59 (1H, J = 7.7 Hz) indicative of a β-configuration for the sugar (Table 2). All other ^{1}H and ^{13}C NMR spectral data were very similar to those of cinnamic esters and 1-substituted β-D-glucopyranoside (Faure et al., 1987). Therefore we established that the unknown substance previously called UN3 (Mouly et al., 1994) was cinnamoyl-β-D-glucopyranoside (CIN) (Table 2).

Although the metabolism of phenolic glucose esters is well-known (Strack and Mock, 1993), the presence of cinnamic acid and its derivatives is not common in plant but considered as a first important intermediate in the biosynthetic pathway of chlorogenic acid (Levy and Zucker, 1960; Hanson, 1966; Kojima and Uritani, 1972a) or a first intermediate of ferulic acid (El-Basyouni and Neich, 1966). Generally *para*-substituted derivatives such as *p*-coumaric acid are encountered. Various different types of structure have been characterized (Kitagawa et al., 1984; Jimenez et al., 1988). The presence of cinnamoyl-β-D-glucopyranoside was suggested in plants (Avadhani and Towers, 1961; Harborne and Corner, 1961; Koukol and Conn, 1961), and it was found and characterized in the roots of sweet potato by Kojima and Uritani (1971, 1972b). The isolation and identification of cinnamoyl-β-D-glucopyranoside for the first time, to our knowledge, in blood orange fruit juices

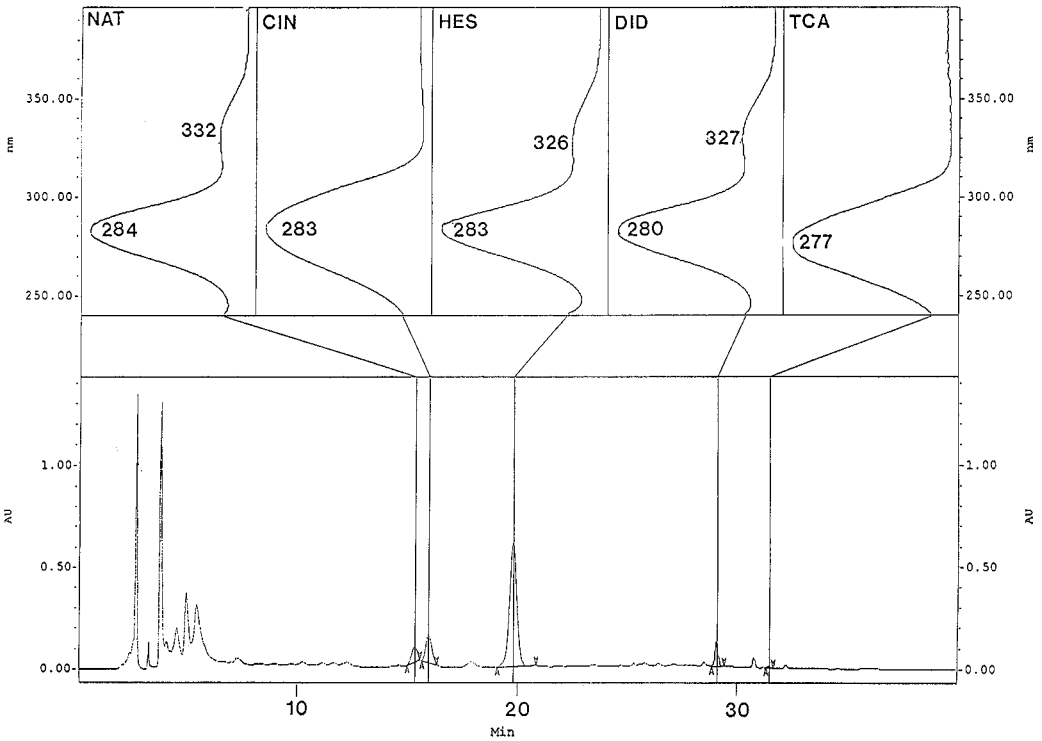


Figure 3. Separation of flavanone glycosides and cinnamic acid derivatives of blood orange juice (var. moro, Italy). For chromatographic conditions, see Figure 2 and Table 1; for peak identification, see Table 3.

Table 4. Flavanone Glycoside and Cinnamic Acid Derivative Compositions in Various Blood Orange Juices Varieties

		compound[a]				
variety		NAT	HES	DID	CIN[b]	TCA
Washington sanguine (WHS)	mean[c]	29	180	11.7	4.0	0.07
	SD[g]	6.1	24.1	2.26	1.12	0.073
	min-max[h]	19−43	129−215	8.0−13.3	2.7−6.4	0.02−0.26
Malta (ML)	mean[d]	38	216	9.7	2.0	0.07
	SD	7.7	45.4	4.40	0.56	0.042
	min-max	25−48	128−286	4.5−20.0	1.1−3.5	0.02−0.13
Sanguinelli (SN)	mean[e]	31	392	8.9	5.7	0.18
	SD	5.4	114.8	3.80	2.03	0.072
	min-max	22−39	259−563	5.6−15.5	2.0−8.7	0.04−0.31
Moro (MO)	mean[f]	42	231	30.9	17.9	0.68
	SD	6.9	22.9	6.00	4.63	0.440
	min-max	29−58	204−279	25.7−47.2	10.9−25.5	0.07−1.81

[a] See Table 3 for name and structural formula. [b] Concentration expressed in mg L^{-1} TCA. [c] Mean (mg L^{-1}) of 12 samples, origin Morocco. [d] Mean (mg L^{-1}) of 12 samples, origin Tunisia. [e] Mean (mg L^{-1}) of 12 samples, origin Spain. [f] Mean (mg L^{-1}) of 11 samples, origin Italy. [g] Standard deviation in mg L^{-1}. [h] Minimum and maximum values in mg L^{-1}.

and in relatively high amounts, affords the opportunity to use this substance as a natural marker for blood orange juice authentication. Accumulation of phenylpropanoids in cotyledons of *Pharbitis nil* strain Violet seedlings during the induction of flowering by poor nutrition was observed (Hairai et al., 1993). Glucopyranosyl sinapate was obtained from rapeseeds (Amarowicz et al., 1995).

Characterization of Some Blood Orange Varieties Using Polyphenol Profiles. The formula and k' of FG, TCA, and CIN, commonly encountered in blood and semi-blood oranges are given in Table 3. The mean of coefficient of variation obtained was less than 1%. The mean of recovery obtained in the FG and TCA was around 95%, and the limit of detection was less than 0.1 mg L^{-1}. Chromatograms of blood orange juices are given in Figure 3. In these chromatographic conditions, CIN is eluted after NAT. In our previous paper (Mouly et al., 1994), the mobile phase contained THF, and this solvent modified the elution power of some flavanone compounds. An inversion of elution order between the CIN and the NAT was observed in absence of THF.

As shown in Table 4 the content in NAT, HES, and DID is not significatively different. Taking into account the TCA and CIN concentrations, the variety differentiation become possible. The Washington sanguine (WHS) were differentiated from the other blood orange varieties by a low HES and DID contents. Malta oranges (ML) were differentiated by the lowest DID, CIN, and TCA contents. Higher concentrations of hesperidin were found in Sanguinelli (SN), which differentiated this variety from the others. In the same way CIN and TCA is present in large amount in this variety. The Moro orange (MO) was differentiated by the highest CIN and TCA contents.

CONCLUSION

Flavanone glycoside determination of four blood orange varieties showed on LC chromatograms the presence in higher concentration of an unknown compound

characteristic of pigmented oranges. This compound was identified as the cinnamoyl-β-D-glucopyranoside, which is an important marker for blood orange juice authentication. Indeed, this compound was not detected in other orange juices. The CIN occurrence associated with FG determination enabled the differentiation of four pigmented orange varieties. Malta and Washington sanguine were characterized by a low FG and cinnamic acid derivatives contents, Sanguinelli by a high HES content and Moro by a high NAT, DID, CIN, and TCA contents.

ACKNOWLEDGMENT

We gratefully acknowledge Mrs. F. Elalmis for technical assistance.

LITERATURE CITED

Amarowicz, R.; Kamarac, M.; Rudnicka, B.; Ciska, E. TLC Separation of glycopyranosyl Sinapate and Other Phenolic compounds. *Fat Sci. Technol.* **1995**, *97*, 330−333.

Avadhani, P. N.; Towers, G. H. N. Fate of Pheylalanine C^{14} and Cinnamic Acid-C^{14} in Malus in Relation to Phloridzin Synthesis. *Can. J. Biochem. Physiol.* **1961**, *39*, 1605−1616.

Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. Distorstionless enhancement of NMR signals by polarization transfer. *J. Magn. Reson.* **1982**, *48*, 323−327.

El-Basyouni, S. Z.; Neish, A.C. Occurrence of Metabolically-Active Bound Forms of Cinnamic Acid and its Phenolic Derivatives in Acatone Powders of Wheat and Barley Plants. *Phytochemistry* **1966**, *5*, 683−691.

Faure, R.; Babadjamian, A.; Balansard, G.; Elias, R.; Maillard, C. Concerted Use of Two-Dimensional NMR Spectroscopy in the Complete Assignment of the ^{13}C and ^{1}H NMR Spectra of Globularin. *Magn. Reson. Chem.* **1987**, *25*, 327−330.

Fisher, J. F.; Wheaton, T. A. A High-Pressure Liquid Chromatographic Method for the Resolution and Quantitation of Naringin and Naringenin Rutinoside in Grapefruit Juice. *J. Agric. Food Chem.* **1976**, *24*, 898−899.

Hairai, N.; Kojima, Y.; Koshimizu, K.; Shinozaki, M.; Takimoto, A. Accumulation of Phenylpropanoids in Cotyledons of Morning Glory Seedlings During the Induction of Flowering by Poor Nutrition. *Plant Cell Physiol.* **1993**, *34*, 1039−1044.

Hanson, K. R. Chlorogenic Acid Biosynthesis. Incorporation of [α-^{14}C] Cinnamic Acid Into the Cinnamoyl and Hydroxycinnamoyl Conjugates of the potato Tuber. *Phytochemistry* **1966**, *5*, 491−499.

Harborne, J. B. *The Flavonoids: Advances in Research Since 1986*; Chapman and Hall: London, 1992.

Harborne, J. B.; Corner, J. J. Plant Polyphenols. IV. Hydroxycinnamic Acid-Sugar Derivatives. *Biochem. J.* **1961**, *81*, 242−250.

Jimenez, C.; Villarde, M. C.; Riguera, R.; Castedo, L.; Stermitz, F. R. Five Phenylpropanoid Glycosides from Mussatia. *Phytochemistry* **1988**, *27*, 2947−2951.

Kamiya, S.; Esaki, S.; Konishi, F. Flavonoids in *Citrus* Hybrids. *Agric. Biol. Chem.* **1979**, *43*, 1529−1536.

Kitagawa, S.; Hisada, S.; Nishibe, S. Phenolic Compounds from Forsythia Leaves *Phytochemistry* **1984**, *23*, 1635−1636.

Kojima, M.; Uritani, I. Isolation of Possible Intermediate of Chlorogenic Acid Biosynthesis in Sweet Potato Root Tissue. *Agric. Biol. Chem.* **1971**, *35*, 632−635.

Kojima, M.; Uritani, I. Studies on Chlorogenic Acid Biosynthesis in Sweet Potato Root Tissue Using *Trans-cinnamic* Acid-2-^{14}C and Quinic Acid-G-^{3}H. *Plant Cell Physiol.* **1972a**, *13*, 311−319.

Kojima, M.; Uritani, I. Elucidation of the Structure of a Possible Intermediate in Chlorogenic Acid Biosynthesis in Sweet Potato Root Tissue. *Plant Cell Physiol.* **1972b**, *13*, 1075−1084.

Koukol, J.; Conn, E. E. The Metabolism of Aromatic Compounds in Higher Plants. IV. Purification and Properties of the Phenylalanine Deaminase of *Hordeum Vulgare*. *J. Biol. Chem.* **1961**, *236*, 2692−2698.

Lee, H. S.; Carter, R. D.; Barros, S. M.; Dezman, D. J.; Castle, W. S. Chemical Characterization by Liquid Chromatography of Moro Blood Orange Juices. *J. Food Compos. Anal.* **1990**, *3*, 9−19.

Levy, C. C.; Zucker, M. Cinnamoyl and *p*-Coumaryl Esters as Intermediates in the Biosynthesis of Chlorogenic Acid. *J. Biol. Chem.* **1960**, *235*, 2418−2425.

Maccarone, E.; Maccarone, A.; Rapisarda, P. Stabilization of Anthocyanins of Blood Orange Fruit juice. *J. Food Sci.* **1985**, *50*, 901−904.

Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: FL, 1990.

Mouly, P.; Gaydou, E. M.; Estienne, J. Column Liquid Chromatography Determination of Flavanone Glycosides in *Citrus*. Application to Grapefruit and Sour Orange Juice Adulterations. *J. Chromatogr.* **1993**, *634*, 129−134.

Mouly, P. P.; Arzouyan, C. R.; Gaydou, E. M.; Estienne, J. M. Differentiation of Citrus Juices by Factorial Discriminant Analysis Using Liquid Chromatography of Flavanone Glycosides. *J. Agric. Food Chem.* **1994**, *42*, 70−79.

Mouly, P. P.; Arzouyan, C. R.; Gaydou, E. M.; Estienne, J. M. Chromatographie des Flavanonosides des Jus de Différentes Variétés de Pamplemousses. Différenciation par Analyses Statistiques Multidimensionnelles. *Analusis* **1995**, *23*, 336−341.

Perfetti, G. A.; Joe, F. L., Jr; Faziot, T.; Page, S. W. Liquid Cromatography for the Characterization of Orange Juice. *J. AOAC Int.* **1988**, *71*, 469−473.

Reminiac, C. C.; Bourrier, M. J.; Goiffon, J. P. Différenciation des Produits de l'Orange et de l'Orange Amére par Analyse Chromatographique des Hétérosides de Flavanones. *Ann. Fals. Experts Chim.* **1989**, *82*, 471−479.

Rouseff, R. L. Differentiating *Citrus* Juices Using Flavanone Glycoside Concentration Profiles. In *Adulteration of Fruit Juice Beverages*; Nagy, S., Attaway, J. A., Rhodes, M. E., Eds.; Marcel Dekker: New York, 1988; pp 49−64.

Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative Survey of Narirutin, Naringin, Hesperidin, and Neohesperidin in *Citrus*. *J. Agric. Food Chem.* **1987**, *35*, 1027−1030.

Saunt, J. *Citrus Varieties of the World*; Sinclair International Limited: Norwich, England, 1990.

Strack, D. *Mock In Methods in Plant Biochemistry*; Lea, P. J., Ed.; Academic Press: London, 1993; pp 45−97.

Viguera, G. C.; Barberan, T. F.; Ferreres, F.; Artes, F.; Lorente, T. F. Determination of Citrus Jams Genuineness By Flavonoid Analysis. *Z. Lebensm.-Unters. -Forsch.* **1993**, *197*, 255−259.

Received for review July 8, 1996. Revised manuscript received October 25, 1996. Accepted October 29, 1996.[⊗]

JF9605097

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1996.