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Estimating Bergamot Juice Adulteration of Lemon Juice by High-Performance Liquid Chromatography (HPLC) Analysis of Flavanone Glycosides

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The chemical composition of 30 samples of juices obtained from bergamot (*Citrus bergamia* Risso and Poit.) fruits is reported and compared to the genuineness parameters adopted by Association of the Industry of Juice and Nectars (AIJN) for lemon juice. It was found that the compositional differences between the two juices are distinguishable, although with difficulty. However, these differences are not strong enough to detect the fraudulent addition of bergamot juice to lemon juice. Instead, we found the high-performance liquid chromatography (HPLC) analysis of the flavanones naringin, neohesperidin, and neoeriocitrin, which are present in bergamot juice and practically absent in the lemon juice, is a convenient way to detect and quantify the fraudulent addition of bergamot juice. The method has been validated by calculating the detection and quantification limits according to Eurachem procedures. Employing neoeriocitrin (detection limit = 0.7 mg/L) and naringin (detection limit = 1 mg/L) as markers, it is possible to detect the addition of bergamot juice to lemon juice at the 1% level. When using neohesperidin as a marker (detection limit = 1 mg/L), the minimal percentage of detectable addition of bergamot juice was about 2%. Finally, it is reported that the pattern of flavonoid content of the bergamot juice is similar to those of chinotto (*Citrus myrtifolia* Raf) and bitter orange (*Citrus aurantium* L.) juices and that it is possible to distinguish the three kinds of juices by HPLC analysis.

KEYWORDS: Bergamot juice; bitter orange; chinotto; lemon juice; flavanone glycosides; fruit juice adulteration

INTRODUCTION

Frauds regarding fruit juices include several aspects, such as the false declaration of the geographic origin of the product, undeclared sugar addition, water addition in "not from concentrate" juices, use of technologies not allowed by legislation (for example, in the case of citrus juices, the addition of peel and/or pulpwash), and the addition of other not declared compounds (such as organic acids, coloring agents, etc.). A rather common kind of fraud in the fruit juice field is the undeclared addition of different fruit juices from botanic-related species (*1*–*4*).

The approach commonly employed by the quality-control laboratories to detect adulterations is generally based on the analysis of chemical and physical parameters, which are then compared to those reported by the Association of the Industry of the Juices and Nectars (AIJN) from Fruits and Vegetables of the European Union. These parameters, estimated on pure juices, are internationally accepted by producers and users of fruit juices and nectars because they were obtained from monitoring and analysis by many international scientific institutions and governmental control agencies (*5*).

Unfortunately, in many cases, especially when the adulteration level is low and accomplished by using other species with similar compositional features, the ability to determine differences with respect to the genuine product by carbohydrate, amino acid, organic acid, and mineral content appears to be more limited in applicability. The usual analytical techniques are often unable to detect the small differences arising from the different origins and production processes of the product to those arising from the fraudulent

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Table 1. Compositional Parameters of Bergamot Juice [a] Compared to the AIJN Reference Guideline for Lemon Juice

	bergamot juice				bergamot juice adjusted to 8.0 °Brix				
compositional parameters	mean	SD	min	max	mean	SD	min	max	lemon AIJN reference guide
soluble solids (%)	9.2	0.5	8.2	10.4					8
L-ascorbic acid (mg/L)	414	26	378	465	360	37	305	454	minimum 150
titration acidity at pH 8.1 (g/L)	44.8	4	38.9	51.5	38.9	3.8	32.2	49.2	44.8−62
D-isocitric acid (mg/L)	423	43	364	531	368	45	318	494	230−500
citric acid/isocitric acid ratio	107	14	80	137	93	13	72	132	maximum 200
L-malic acid (g/L)	1.6	0.5	0.9	2.9	1.4	0.5	0.7	2.5	1.00−7.05
formol number	24.2	3.1	19.9	30.1	21.1	3.2	16.4	26.1	13−26
ash (g/L)	3.2	0.23	2.81	3.61	2.78	0.20	2.48	3.37	2.02−4.03
sodium (mg/L)	17	5	9	26	15	4	8	24	maximum 30
potassium (mg/L)	1359	109	1169	1567	1181	114	992	1441	1100−2000
magnesium (mg/L)	92	17	67	123	80	15	58	113	70−120
calcium (mg/L)	76	12	57	98	66	12	49	89	45−160
sugars (g/L)									
glucose	12	2	9	14	10	1	8	13	3−12
fructose	12	2	9	15	10	1	8	13	3−11
sucrose	17	4	10	24	15	3	8	20	maximum 7.00
glucose/fructose	1	0.04	0.94	1.06	1.00	0.04	0.94	1.06	0.95−1.03
water-soluble pectins (mg/L)	308	28	247	361	268	28	206	314	maximum 700
amino-acids (mg/L)									
L-aspartic acid	222	10	201	241	193	14	170	223	300−800
L-glutamic acid	166	10	144	182	144	11	119	168	160−400
L-serine	180	5	169	194	156	11	139	180	135−370
L-asparagine	84	4	77	91	73	5	64	83	130−600
L-glycine	7	1	7	8	6	1	5	7	7−25
L-glutamine	23	1	22	25	20	1	18	23	maximum 45
L-histidine									maximum 10
GABA	66	3	61	70	57	4	51	66	60−185
L-threonine	29	5	21	36	25	4	17	34	10−30
L-alanine	84	6	74	97	73	7	61	90	80−260
L-arginine	30	4	21	35	26	4	18	31	maximum 100
L-proline	230	6	219	243	200	13	177	226	100−800
L-tyrosine	6	1	5	7	5	0	4	6	maximum 7
L-valine	12	2	9	19	10	2	8	18	8−35
L-methionine									maximum 5
L-isoleucine	6	1	4	8	5	1	3	7	3−10
L-leucine	8	2	5	12	7	2	4	11	3−10
L-phenylalanine	19	3	13	29	16	3	12	24	8−40
L-lysine	10	1	7	12	9	1	6	11	5−20
flavonoids HPLC (mg/L)									
neoeriocitrin	121	22	64	164	105	18	57	140	
eriocitrin	8	3	5	12	7	2	4	11	
narirutin	14	8	5	34	12	7	4	26	
naringin	106	22	68	147	92	19	57	125	
hesperidin	<5	<5	<5	<5	<5	<5	<5	<5	200−800
neohesperidin	64	14	41	98	56	13	36	86	
flavonoids according to Davis (mg/L)	547	39	480	615	476	40	397	540	maximum 1500

[a] The reported data are the mean values of the 30 lots analyzed in the 3 harvest years.

addition of juices with similar composition. For these reasons, in recent years, besides the traditional analytical approaches, two strategies have been added for a better definition of the "purity" and/or the "authenticity" of the fruit juices, the analysis of stable isotopes (*6*) and the characterization and analysis of minor components (natural products) present in the juices, particularly flavonoids, which can be employed to identify both juices and their adulteration (*7–9*).

The analysis of stable isotopes has revealed a particularly efficient technique to determine sugar and water addition to fruit juices. However, this technique is of limited use because of the requirement of specialized staff and equipment costs, which is out of the reach of normal quality-control laboratories. On the contrary, the analysis of minor components is of wider applicability and much less expensive. In this respect, flavonoids are of great interest for citrus juice characterization because many papers have contributed to the detailed definition of their distribution in the many juices (*10, 11*). As a consequence, these compounds can be used as markers for the control and certification of citrus juices.

The bergamot (*Citrus bergamia* Risso and Poit.) is a fruit produced almost exclusively in the Calabria region (southern Italy) and, specifically, in a narrow zone of the province of Reggio Calabria and contributes more than 95% of the bergamot essential oil world production, the most valuable of the citrus essential oils.

The geographic origin of bergamot orange is still uncertain as was for many years its botanic classification. Only recently have some authors determined its origin (*12*) through genetic marker analysis, such as random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR). It was demonstrated that bergamot, which was already present in Calabria at the beginning of 19th century, is a hybrid of citron (*Citrus medica* L.) and bitter orange (*Citrus aurantium* L.).

The lack of interest in the Italian citrus industry for bergamot juice production is paralleled by the scarcity of studies on its composition. As a matter of fact, the literature is rich in papers on its essential oil composition, but the juice obtained from bergamot is probably the least studied among the citrus juices, with only limited information present in the literature (*13–18*).

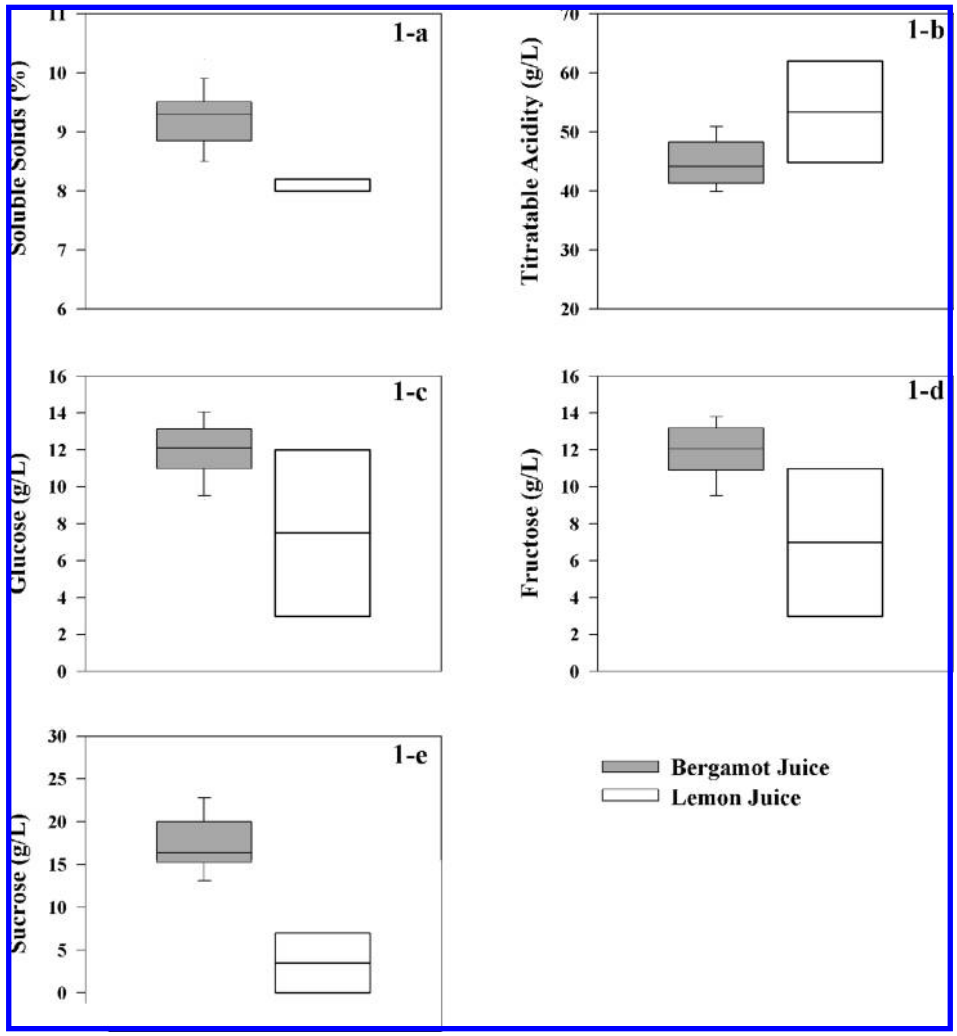

Figure 1. Boxplot comparisons of bergamot juice and lemon juice parameters from AIJN reference guideline.

As for production, the harvesting and successive transformation of bergamot fruits begin in November and continue until March. The mean yield of fruit is about 15−20 tons/hectare, and the yield in essential oil is about 5 kg/ton. The most important byproduct of the bergamot essential oil industry is the juice, which has excellent organoleptic features, notwithstanding a moderate bitter aftertaste. Unfortunately, because of the lack of marketing, the rather limited bergamot juice production, and the absence of reference parameters (AIJN) for its use, fraudulent operators use bergamot juice to adulterate other citrus juices that have a wider market. In particular, lemon juice, which has color and aroma very close to bergamot juice, is the most suitable target for this kind of fraud (*19*).

For this reason, we thought finding an easy analytical method to assess lemon juice genuineness to be of interest and exploring the fraudulent addition of bergamot juice to lemon juice through the analysis of juice flavonoid components.

MATERIALS AND METHODS

Reagents and Standards. Acetonitrile for high-performance liquid chromatography (HPLC) and *N*,*N*-dimethylformamide were from Sigma-Aldrich. The flavonoids neoeriocitrin, eriocitrin, narirutin, naringin, hesperidin, neohesperidin, quercetin, and diosmin were from Sigma-Aldrich and Extrasynthes (France). The standard mixture of L-amino acid containing Ala, Arg, Asp, Glu, His, Iso, Leu, Lys, Met, Phe, For, Ser, Thr, Tyr, Val, and Cys at 2.5 mM concentration in HCl was from Pierce. L(+)-ascorbic acid sodium salt and D(+)-galacturonic acid were from Fluka BioChemika. Nitric acid (65%), sulfuric acid (98%), glacial acetic acid, and carbazole were from Carlo Erba Reagents. All other solvents and reagents were of analytical grade.

Bergamot Juice: Sample Preparation and Analytical Determinations. To obtain a large number of data on the composition of bergamot juice, 30 lots, each of 3 kg of fruits harvested in the month of January from the production years 2003−2005, were used. The analyses were conducted year after year. The bergamot lots were undifferentiated as far as the cultivars are concerned. As a matter of fact, three bergamot cultivars are grown in the Calabria region, i.e., "Femminello", "Castagnaro", and "Fantastico". However, the "Fantastico" cultivar production predominates for more than 80%.

After of the fruits were exhaustively washed, the juice, obtained using a manual citrus-fruit squeezer, was filtered through a stainless-steel filter with a mesh diameter of 1.18 mm, stored in 50 mL aliquots in plastic bags, and immediately frozen at −20 °C.

The following determinations were conducted in triplicate on all of the samples according to the International Federation of Fruit Juice Producers (IFU) methods (*20*) of the International Association of the juices and nectars of fruit (AIJN): soluble solids, expressed in °Brix, according to IFU procedure number 8; total acidity, expressed as citric acid monohydrate, according to IFU procedure number 3; formol number, expressed as milliliters of 0.1 N NaOH for 100 mL of juice, according to IFU procedure number 30; ascorbic acid, expressed in mg/L, determined by titration with 2,6-dichloroindophenol according to IFU method number 17.

Ashes, expressed as g/L, were determined according to IFU method number 9.

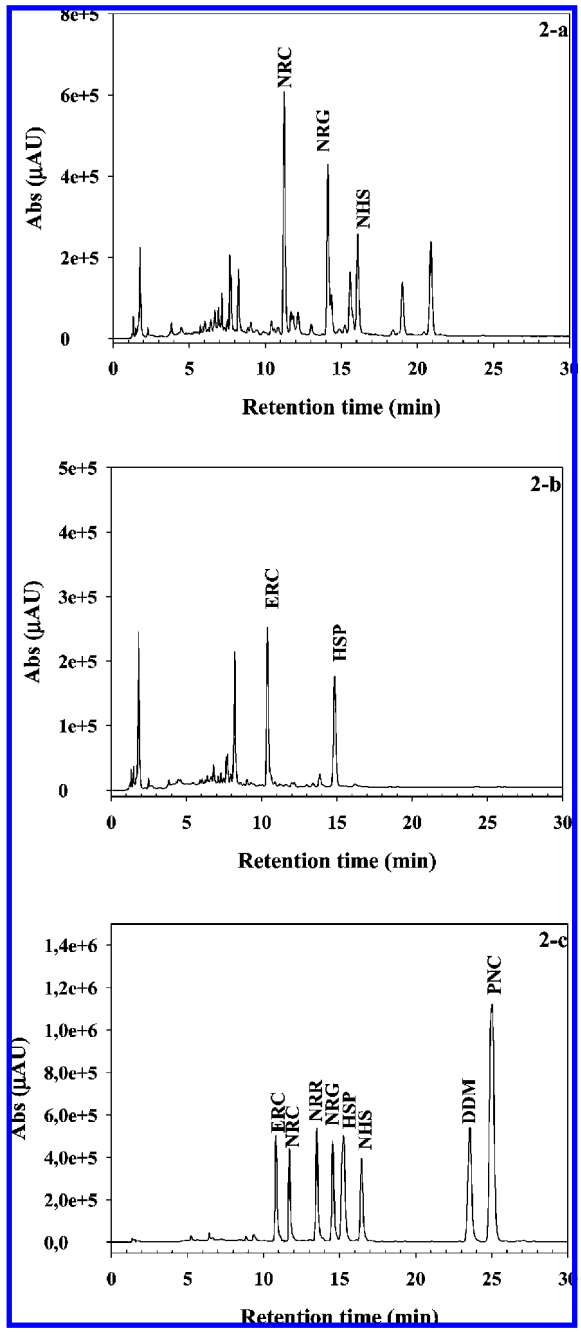

Figure 2. Flavonoid patterns of the (a) bergamot juice and (b) lemon juice. (c) Standard mixture contained eriocitrin (ERC), neoeriocitrin (NRC), narirutin (NRR), naringin (NRG), hesperidin (HSP), neohesperidin (NHS), didymin (DDM), and poncirin (PNC) shown here at the 50 mg/L concentration. The absorption wavelength was set at 287 nm.

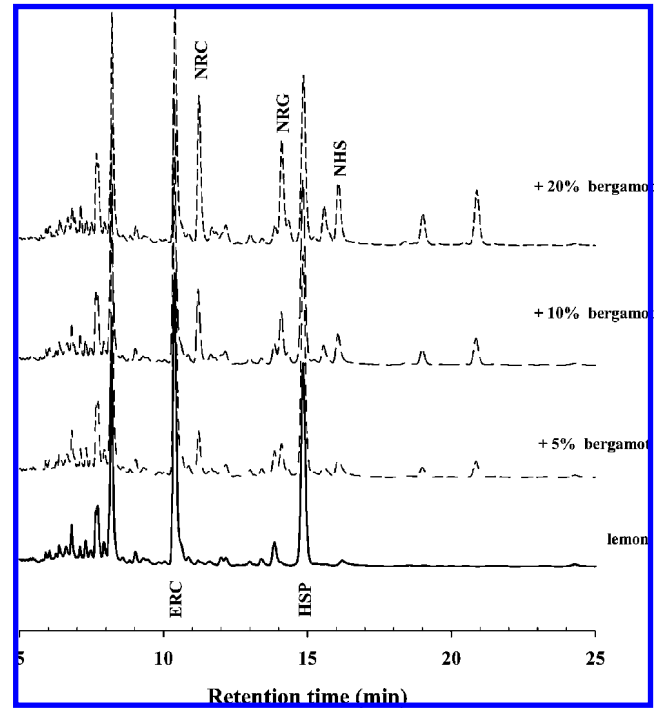

Figure 3. HPLC pattern of the lemon juice spiked with increasing amounts of bergamot juice: eriocitrin (ERC), neoeriocitrin (NRC), naringin (NRG), hesperidin (HSP), and neohesperidin (NHS). The absorption wavelength was set at 287 nm.

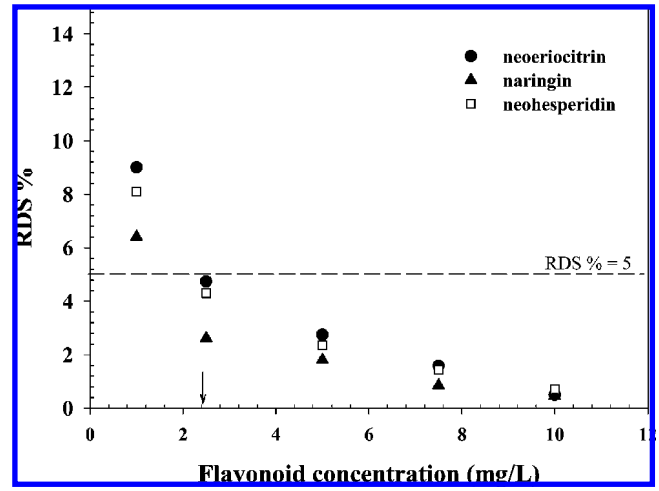

Figure 4. Relative percentage standard deviations for neoeriocitrin, neohesperidin, and naringin added to the lemon juice at various concentrations to calculate the LOQ.

Glucose, fructose, and sucrose were determined according to IFU method numbers 55 and 56, respectively, L-malic acid according to IFU method number 21, D-isocitric acid according to IFU method number 54, and the hydrosoluble pectins according to IFU method number 26. The total flavonoid content was determined in all of the samples as indicated by AIJN according to the Davis method (*21*). As for metal content, the determinations of sodium, potassium, calcium, and magnesium were carried out after mineralization of the samples according to Metodi Ufficiali di Analisi per le Conserve Vegetali (MUACV) (*22*) and inductively coupled plasma atomic emission spectrometry (ICP−AES) (*23*) procedures.

Flavonoid Analysis by HPLC. The determination of the flavonoids in the juices has been carried out by liquid chromatography according to the method of Grandi et al. (*7*), employing a ThermoFinnigan Surveyor HPLC system equipped with a diode array detector interfaced to a Dell computer Optlex gx260, and using a Xcalibur software for the signal acquisition and elaboration. Standard solutions of the flavonoids neoeriocitrin, eriocitrin, narirutin, naringin, hesperidin, neohesperidin, didymin, and poncirin were prepared by weighing exactly 0.1 g of each compound and dissolving it in 100 mL of *N*,*N*-dimethylformamide. Those solutions were used to build up the calibration lines by diluting them to cover the concentration range of 10−100 mg/L.

For the flavonoid analysis, the juices (10 mL) were shaken with 20 mL of a 1:1 (v/v) mixture of 0.25 M *N*,*N*-dimethylformamide/ammonium oxalate and 20 mL of analytical-grade water and then filtered on 0.45 μm PTFE Pall filters. A volume of 5 μL was employed for the HPLC analysis on a Phenomenex Luna column C18 (l50 × 3 mm) 5 μm. The elution was conducted as indicated by Grandi et al. (*7*). The eluent A was made by an aqueous solution of 5 mM KH_2PO_4 adjusted at pH 3.05 with phosphoric acid. The eluent B was obtained by mixing acetonitrile/water/0.25 M KH_2PO_4, in the ratio 70:26:4

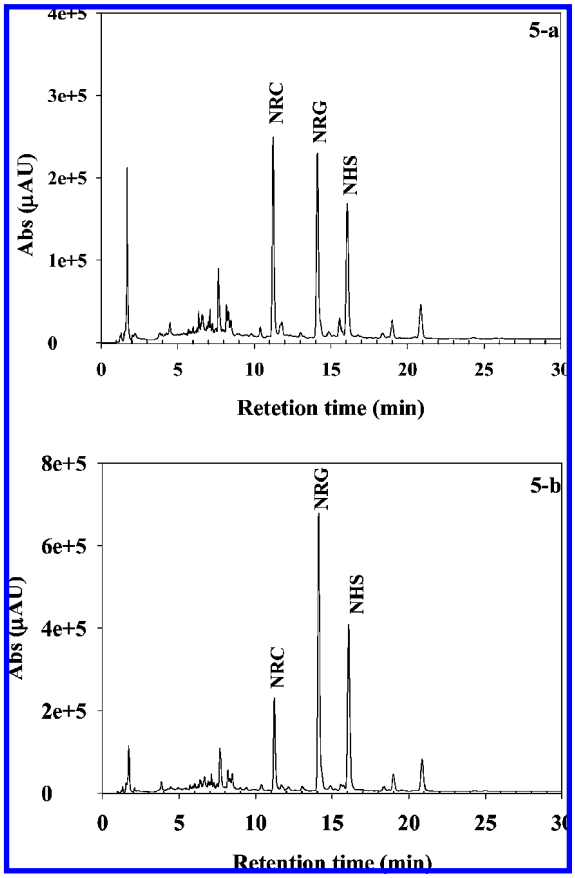

Figure 5. HPLC patterns of chinotto (a) and bitter orange (b) juices: neoeriocitrin (NRC), naringin (NRG), and neohesperidin (NHS). The absorption wavelength was set at 287 nm.

(v/v/v), and adding 100 μL of H_3PO_4 (87%) per liter of solution. The identification of the flavonoids was based on retention time, and quantification was achieved by external standard calibration.

HPLC Analysis of Amino Acids. The amino acid determinations were performed by the Waters AccQ-Tag method as described by Cohen et al. (*24*). The reversed-phase (RP)-HPLC analysis was made employing a Waters chromatograph mod. 2690 equipped with a Waters fluorescence detector mod. 474 and a postcolumn derivatization system. The analytical conditions were excitation wavelength, 250 nm; emission wavelength, 395 nm; column AccQ-Tag, 3.9 × 150 mm, thermostatted at 37 °C; and flow rate, 1 mL/min. Identification and quantification of the amino acids were carried out by a comparison to a calibration line obtained by employing an amino acid standard mixture (Pierce) at a known concentration.

Determination of the Adulteration Level (as a Percentage of Added Bergamot Juice) in Lemon Juices. To assess a reliable estimate of the adulteration level as a consequence of fraudulent addition of bergamot juice to lemon juice and its detection limit, lemon juices with increasing additions of bergamot juice were prepared and analyzed by HPLC as reported above. The method was validated according to the Eurachem rules (*25*). In brief, adulterated lemon juices were prepared by adding 5, 10, 20, 30, and 50 mL of bergamot juice to lemon juice to a final volume of 100 mL. The samples (prepared in triplicate) were analyzed by HPLC as described above for the flavonoid content of naringin, neohesperidin, and neoeriocitrin, which are normally absent in the pure lemon juice. The results were correlated with the amount of bergamot juice added.

LOD and LOQ Determinations. To estimate the detection and quantification limits for naringin, neohesperidin, and neoeriocitrin, 10 samples of lemon juice, which represented the blanks, were analyzed as reported above. The detection limits (LOD) for these flavonoids were assumed as 3 times the standard deviation of the instrumental response recorded at their retention times. As far as the limit of quantification (LOQ) is concerned, five lemon juice samples spiked with naringin, neohesperidin, and neoeriocitrin to final concentrations of 1, 2.5, 5, 7.5, and 10 mg/L were analyzed. Six chromatographic analyses were performed, according to the Eurachem procedures (*25*), on each sample, and the relative percentage standard deviation (RSD%) for each set of analyses was calculated for each flavonoid. The calculated RSD% was plotted against the amount of adulteration in the juice. The LOQ value was assumed as the lowest analyte concentration that can be determined with a RSD lower than 5%.

Statistical and Graphic Elaborations. The comparison boxplot of the data from bergamot and lemon juices and the canonical discriminant analysis to differentiate among bergamot, bitter orange, and chinotto juices were performed using the statistical program SPSS rel.10.0.5 for Windows.

RESULTS AND DISCUSSION

The values of the main compositional parameters obtained from the analyses of 30 samples of bergamot juices are summarized in **Table 1**. It is worth noting that the main juice compositional parameters did not significantly vary among the analyses conducted in the 3 years (2003−2005) (data not shown). Moreover, they resulted very close to those reported in a previous study (*16*) on bergamot juices obtained with different extractive procedures. However, the flavonoid contents in the juices obtained with industrial procedures were slightly higher, although their relative ratios did not change (*16*).

The data of **Table 1** were compared to the parameters codified for lemon juice by AIJN (*5*). Bergamot juice shows a soluble solid content somewhat higher than the standard value for lemon juice (**Figure 1a**). The simple sugar content is also higher (parts **c**−**e** of **Figure 1**). In particular, the high sucrose content of bergamot juice can reach values greater than 2%, while the maximum value proposed for lemon juice is 0.7% (**Figure 1e**). These differences are probably due to acidity (**Figure 1b**), generally higher in the lemon juice, which may contribute to the hydrolysis of sucrose into glucose and fructose.

A significant difference between the two juices is also seen for the formol number that is higher for bergamot juice. Surprisingly, this does not apply to the amino acid content. In fact, the amino acids present in the two juices, although the same, have lower concentrations in bergamot juice. These differences (higher formol number and lower amino acid content) in bergamot juice may be due to the amino group containing substances, which are not free amino acids, contributing to the formol number. All of the other parameters examined were about the same for the two juices.

Although the compositional differences between the two juices are in some cases relevant, they are essentially quantitative and unsuitable to detect and quantify with sufficient reliability the addition of one juice to the other when taking into account the naturally occurring compositional variability, which depends upon numerous factors. Instead, analysis of juice flavonoid components provides a better basis for this aim. Many authors report that flavonoids are not only differentially distributed in the various parts of the citrus fruits but have patterns characteristic to botanic species. Therefore, they represent a good analytical target for detecting the presence of one juice in another, even in the case of closely related species (*1, 3*). To this aim, the four neohesperidose flavonoids (neohesperidin, naringin, neoeriocitrin, and poncirin) and the main four rutinose flavonoids (hesperidin, narirutin, eriocitrin, and didymin), for which the variability in many citrus species is known, appear particularly suitable for citrus products. Other flavonoids are less suitable because of the lack of such data and because their presence in citrus products is much lower, which decreases the ability to determine adulteration based on analysis and quantification.

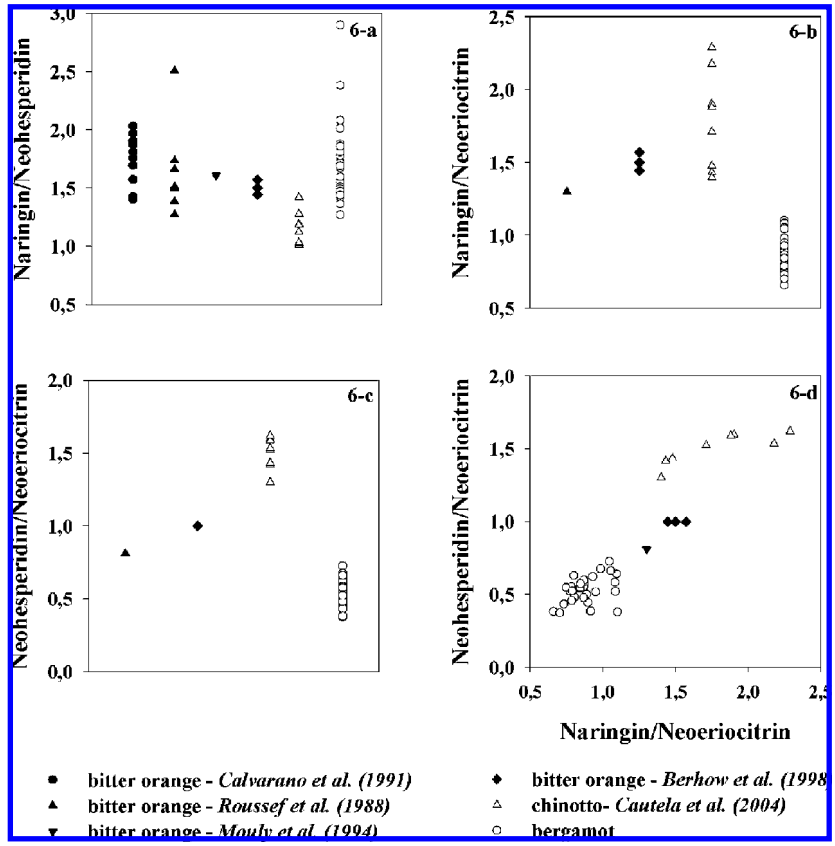

Figure 6. Ratios of neohesperidose flavonoid contents in bitter orange and chinotto juices. The data gathered from the indicated references are compared to the analogous ratios for bergamot juice.

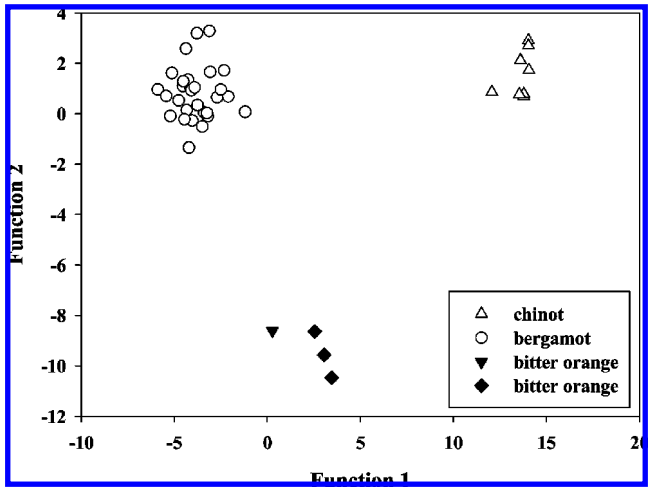

Figure 7. Scatterplot of the three cluster groupings on the two canonical discriminant functions. The data of the neohesperidose flavonoids gathered from the reported references are compared to the analogous data for the bergamot juice (○). Bitter orange (◆), data from Berhow et al. (*30*); bitter orange (▼), data from Mouly et al. (*8*); and chinotto (△), data from Cautela et al. (*29*). The calculated discriminant functions were function 1 = −26.68 + 0.014NRC − 0.002NRG − 0.01NHS + 8.425NRG/NHS − 13.994NRG/NRC + 37.688NHS/NRC and function 2 = 3.063 − 0.058NRC − 0.039NRG + 0.081NHS + 3.405NRG/NHS − 1.545NRG/NRC − 1.219NHS/NRC, where neoeriocitrin (NRC), naringin (NRG), and neohesperidin (NHS).

The typical flavonoid pattern of bergamot juice is shown in **Figure 2a**, and the experimental values determined in lemon and bergamot juice are reported in **Table 1**. It clearly appears that bergamot juice is characterized by noticeable amounts of neohesperidose flavonoids (naringin, neoeriocitrin, and neohesperidin) and lower amounts of rutinose flavonoids (narirutin, hesperidin, eriocitrin, and didymin; data not shown). The data suggest some considerations. The first is that, from an organoleptic point of view, the elevated presence of neohesperidose flavonoids and the lower presence of rutinose flavonoids explain the pleasant nuance of bitter taste of the bergamot juice. It is known, in fact, that glycosylation not only makes the flavanone more soluble but it also influences the taste. When the flavanone ring is glycosylated with neohesperidose (IV) (rhamnosyl-α-(1 → 2) glucose), the resulting flavonoid is markedly more bitter (naringin, poncirin, neohesperidin, and neoeriocitrin are all bitter flavonoids). When the glycosylation is due to rutinose (V) (rhamnosyl-α-(1 → 6) glucose), the resulting flavonoids are tasteless, such as hesperidin, eriocitrin, didymin, and narirutin (*26*).

The second consideration is that, from an analytical point of view, the reported data are in good agreement with those already reported by others (*15*–*17*). Nogata et al. (*18*) reported the flavonoid composition in the albedo, flavedo, epidermis segment, and juice vesicle tissue of 42 species and cultivars of the *Citrus* genus, two *Fortunella* species, and one *Poncirus* species. For bergamot, these authors found a flavonoid pattern in juice vesicles very similar to what we found in the juice. However, they reported a high concentration of the flavonoid poncirin, which we did not find in any of the bergamot juice samples that we analyzed.

More interestingly, the different flavonoid patterns of bergamot and lemon juices (parts **a** and **b** of **Figure 2**) allow for detection of the addition of bergamot juice to lemon juice. In fact, the flavonoids naringin, neohesperidin, and neoeriocitrin are practically absent from lemon juice. Therefore, the fraudulent addition of bergamot juice can be easily revealed by the presence of these compounds. Lemon juice is characterized almost

exclusively by the presence of the two rutinose flavonoids, eriocitrin and hesperidin, which are absent in bergamot juice, and by minor amounts of narirutin, didymin, and the flavone diosmin. It has been reported by Grandi et al. (*7*) and successively by Robards et al. (*27*) and Caristi et al. (*28*) that eriocitrin is the more characteristic flavonoid of lemon juice, and therefore, it must always be present. The hesperidin content determined by HPLC is lower than the content found with the Davis method (*21*) and varies in the range of 200−800 mg/L for cloudy juices. These parameters for lemon juices are accepted and codified by the AIJN.

As an example, the chromatograms of some lemon juices spiked with increasing amounts of bergamot juice are reported in **Figure 3**. The increase in the three neohesperidose flavonoids neoeriocitrin, naringin, and neohesperidin after successive additions of the bergamot juice is evident. To estimate the minimum percentage of detectable adulteration, the limits of detection (LOD) and quantification (LOQ) for the flavonoids naringin, neohesperidin, and neoeriocitrin were determined directly in the lemon juice matrix, which was used as a blank, as described in the Material and Methods. The detection limits turned out to be 0.7 mg/L for neoeriocitrin and 1 mg/L for naringin and neohesperidin. As shown in **Figure 4**, the LOQ, assumed as the lowest analyte concentration that can be determined with a RSD lower than 5%, was about 2.5 mg/L. On the basis of these results, it was possible to detect the lowest addition of bergamot juice to the lemon juice at a 1% level, employing as markers neoeriocitrin and naringin. When neohesperidin was used as a marker, the lowest detectable addition level was about 2%.

The presence of the neohesperidose flavonoids in the lemon juice is ascribable to adulteration, because such compounds are normally absent in the lemon juice. However, it does not unequivocally indicate the adulteration source. In fact, the flavonoid pattern of the chinotto and bitter orange juices, reported in parts **a** and **b** of **Figure 5**, show remarkable similarity with that of bergamot juice (*29*), with some differences arising only from the relative amounts of the neohesperidose flavonoids.

It is difficult to determine from a neohesperidose flavanone pattern in a lemon juice sample if it was adulterated with bergamot chinotto and/or bitter orange juices, although chinotto and bitter orange juice adulteration is unlikely because both chinotto and bitter orange juices are much less available then bergamot juice. However, we thought it useful to verify this possibility and to find some analytical parameters to solve this problem using literature data on the flavanone content of chinotto and bitter orange juices and our bergamot juice data.

For the flavanones in chinotto juice, we found only the analytical data recently reported by Cautela et al. (*29*). On the contrary, for orange juices, recent critical reviews by Peterson et al. (*10, 11*) report flavanone contents in many citrus fruits, including bitter orange (*C. aurantium*) [data from Calvarano et al. (*13*), Mouly (*1*), and Berhow et al. (*30*)]. To differentiate between the addition of bergamot juice, bitter orange, and/or chinotto juices, we calculated the naringin/neohesperidin ratios from the analytical data found in the cited references and compared those ratios to analogous ratios from bergamot juice analytical data (**Figure 6a**). The ratio naringin/neohesperidin, as shown in **Figure 6a**, is essentially the same for the three juices; therefore, it cannot be a discriminating parameter for these juices. Instead, we have found that the comparison of the ratios naringin/neoeriocitrin and neohesperidin/neoeriocitrin (**Figure 6d**) supplies with remarkable reliability the ability to discriminate between the addition of bergamot juice to lemon juice or that of chinotto juice to lemon juice. In other words, when the ratio naringin/neoeriocitrin is comprised between 0.6 and 1.1 and the ratio neohesperidin/neoeriocitrin is comprised between 0.4 and 0.8, the lemon juice adulteration can be ascribed with sufficient reliability to the bergamot juice (parts **b** and **c** of **Figure 6**). On the other hand, for values of the ratio neohesperidin/neoeriocitrin higher than 0.8 and for values of the ratio naringin/neoeriocitrin higher than 1.3, the juice adulteration is presumably due to bitter orange and/or chinotto juice. Unfortunately, because the values of those ratios is similar, the comparison is not discriminating between bergamot juice and bitter orange juice additions.

More reliable conclusions with respect to the comparison of these ratios can be made by performing a canonical discriminant analysis, where the relative amounts of the flavonoids neoeriocitrin, naringin, and neohesperidin and the ratios naringin/neohesperidin, naringin/neoeriocitrin, and neohesperidin/neoeriocitrin are used to find the two discriminant functions that more reliably indicated which of the three juices was used to adulterate the lemon juice (**Figure 7**).

In conclusion, the HPLC analysis of naringin, neohesperidin, and neoeriocitrin, characteristic flavanones of the bergamot juice and absent in the lemon juice, can be used effectively to determine and quantify the fraudulent addition of bergamot juice to lemon juice above an adulteration level of 1%. Moreover, the canonical discriminant analysis can indicate if the lemon juice adulteration was with chinotto or bitter orange juice, although such an occurrence is unlikely due to the limited availability of these juices.

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