JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Genotype and Harvest Time Influence the Phytochemical Quality of Fino Lemon Juice (*Citrus limon* (L.) Burm. F.) for Industrial Use

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Two clonal selections of lemon tree (*Citrus limon* Burm. f. cv. Fino), named Fino-49-5 and Fino-95, were studied to ascertain the influence of genetic (clone) and environmental (season) factors on the human-health bioactive compounds of lemon juice (vitamin C and flavonoids) and the possible relationship between composition and in vitro antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid), and ferric reducing antioxidant power) of the juice. The cultivar Fino-49-5 performed better in terms of flavonoid and vitamin C contents. Variability in the weather conditions determined, at least in part, differences in the content of lemon juice bioactives more importantly than the genetic background did. Therefore, the food industry would have phytochemically rich and nutritive lemons with practically complete independence of the harvest time and the selected cultivar.

KEYWORDS: Lemon juice; citrus fruit; flavonoids; vitamin C; antioxidant; season

INTRODUCTION

Eating a healthy diet has become an important part of everyday life. In this sense, fruits and vegetables are a very important part of a balanced diet, particularly because of their role in the prevention of diseases (e.g., obesity and diabetes) (*1*) and certain types of cancer (*2, 3*). Lemon (*Citrus limon* (L.) Burm. f) is a rich source of nutrients, including flavonoids, citric acid, vitamin C, and minerals (*4, 5*). Traditionally, the health-promoting properties of citrus fruits and juices, lemon fruits in particular, have been attributed to their vitamin C contents, but recently, it has been shown that flavonoids may have antioxidant (*6*), antimutagenic (*7*), anti-inflammatory, antiallergic, antiviral, antiproliferative, cardioprotective (*8–11*), and anticarcinogenic effects (*2, 3*) and blood-lipid lowering properties (*12–14*).

Citrus species have undergone numerous genetic modifications because of the frequent mutations that they undergo spontaneously, sporadic hybridizations, and natural selection (*15*). Lemon production in Murcia (southeastern Spain) represents more than 95% of the citrus production in Spain (*16*), the cultivars Fino and Verna being the predominant lines (*17*). Fino lemons are of high quality for international exports to European countries from October to February when prices are high (*17*).

The analyses of quality parameters of lemon juices are of special interest (Codex Alimentarius, European Comission). In this sense, lemon juice could be used as a substitute for citric acid (E330), which is widely used as legal additive in beverages and as acidifier and preservative in fruit juices, jellies, confitures, and organic and environmentally friendly foods. These features open new ventures for producers and processing companies of lemon fruits in food industry. Moreover, Spain is the third lemon-producing country in the world and the first in Europe, producing 1 130 000 tons (*18*); a total of 552 000 tons are exported, and 212 000 tons are used in industrial processing. Therefore, overproduction is favorable.

Concerning the human-health-related phytonutrients in lemon juice, hesperidin and eriocitrin (flavanones), together with small amounts of diosmetin glycosides (flavones) (*19*), are the main compounds (*20*). Other flavonoids identified in lemon juice are vicenin-2 (*19, 20*), iso/limocitrol 3-β-glucoside, and limocitrin 3-β-glucoside (*9, 19*). Quercetin and myricetin (*20, 21*), as well as other phenolic compounds such as hydroxyl-cinnamic acids (*19*), are also present in very low concentrations.

The present study compared two clones of Fino lemon trees (Fino-49-5 and Fino-95), established in Murcia (southeastern Spain; Mediterranean semiarid climate), and was aimed at characterizing the composition of their juices and finding possible relationships between phytochemical composition and antioxidant biological properties. Although there is a wealth of evidence on lemon-tree rootstocks and production quality (*22*) and many works on agronomic aspects of lemon trees (*23*), knowledge on the effects of genotype and environmental factors on the quality of Fino lemon clones for industrial purposes is needed.

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10.1021/jf073282w CCC: $40.75
Published on Web 02/07/2008

Table 1. Climatic Conditions of the Fruit Tree Orchards through the Experimental Period (2004–2006)[a]

	2004			2005			2006		
	E	M	L	E	M	L	E	M	L
T (°C)	13.8	9.7	12.7	13.8	10.7	16.3	13.8	14.1	14.2
RH (%)	62.9	60.8	63.0	63.8	64.2	54.3	63.8	61.8	54.1
solar ($W \cdot m^{-2}$)	110.7	138.0	184.2	114.2	125.5	203.8	114.2	132.8	184.8
rainfall (mm)	25.6	41.3	5.5	33.9	16.3	1.3	33.9	9.0	39.1

[a] E, early sampling (November); M, medium sampling (February); L, late sampling (March); *T*, temperature (°C); RH, relative humidity; solar: solar radiation flux.

Table 2. Average Quality Parameters of the Juice Extracted from Fino-49-5 and Fino-95 Lemons (2004–2006)[a]

	Fino-49-5			Fino-95			LSD
	E	M	L	E	M	L	($n = 12$)
TSS[b]	9.83 a	9.02 b	8.35 c	9.12 b	8.99 b	8.25 c	(0.58)***
pH	2.34b c	2.49 a	2.51 a	2.35 b	2.48 a	2.31 c	(0.04)***
TA[c]	7.14 a	6.61b c	6.07 d	6.80 b	6.35 cd	5.48 e	(0.26)***
juice yield[d](%)	24.65 e	29.01 cd	29.81 bc	26.54 de	33.22 a	32.04 ab	(23.02)***

[a] Means ($n = 4$) in the same row followed by different letters are significantly different at $P < 0.05$ according to Duncan's test. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$. [b] TSS is expressed as °Brix (20°C). [c] TA (titratable acidity) is expressed as grams of citric acid per 100 mL. [d] Juice yield from 2004-season is not included. Juice yield is expressed as percentage (w:w) ($n = 8$).

MATERIALS AND METHODS

Fruit Tree Orchards. The experiments were replicated during three consecutive years (2004–2006). Lemon trees (*Citrus limon* Burm. f. cv. Fino) were grown at the CEBAS-CSIC's experimental farm (La Matanza, Santomera, Murcia, southeastern Spain). The experimental site (38°6′14″ N, 1°1′59″ W) has a clay−loam soil profile (paralithic mollic-calciorthid). Available soil–water and bulk density were 106 $mm \cdot m^{-1}$ and 1.5 $mg \cdot m^{-3}$, respectively. Saturated hydraulic conductivity was 8.0 $cm \cdot h^{-1}$. The experiments were carried out on 14-year-old lemon trees. Tree spacing was 6 × 6 m, with an average ground cover of about 65%. Environmental conditions of the harvest dates are presented in **Table 1**.

Full production period of Fino trees through the three years studied (2004, 2005, and 2006) extended from early sampling (E) in November, through medium sampling (M) in February, to late sampling (L) in March. The M in 2004 could not be performed because of damaging frost in the area; data are not available for analysis (*24*).

Sample Preparation. Lemon juice was obtained from five lemons from four lemon trees of Fino-49-5 and Fino-95 clones. Analyses and tests were carried out in duplicate. A domestic squeezer (Citromatic, Braun Española S.A., Barcelona, Spain) was used for the preparation of the juices, which was realized by carefully squeezing the fruits by hand to avoid contamination by components in albedo. Samples of freshly prepared juice were kept frozen (−20 °C) for further analyses.

Extraction of Phenolic Compounds. All samples were centrifugated 5 min at 10 500*g* (model Sigma 1-13, B. Braun Biotech International, Osterode, Germany) at 4 °C. The supernatant (soluble fraction) was filtered through a 0.45 μm filter (Millex HV13, Millipore, Bedford, MA) before injection into the HPLC.

Reagents and Standards. Ascorbic acid (AA), dehydroascorbic acid (DHAA), 2,2-diphenyl-1-picryl-hydrazyl (DPPH•), and cetyltrimethylammonium bromide (cetrimide) were purchased from Sigma-Aldrich (Steinheim, Germany); formic acid, methanol, all of analytical grade, and hesperidin were purchased from Merck KGaA (Darmstadt, Germany); diosmin was purchased from Genay (France); 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS chromophore) was purchased from Calbiochem, Merck KGaA (Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and 1,2-phenylenediamine dihydrochloride (OPDA) were purchased from Fluka Chemika (Neu-Ulm, Switzerland); gallic acid was purchased from Doesder. Chem. Co. (Barcelona, Spain); and potassium dihydrogen phosphate was purchased from Panreac Química S.A. (Barcelona, Spain). Milli-Q water was produced by using an Elix 3 Millipore water purification system (Molsheim, France)).

HPLC Analysis, Identification, and Quantitation of Flavonoids. The method previously reported by Pérez-Vicente et al. (*25*) was followed for identification and quantitation of flavonoids. Each sample was analyzed on a Merck-Hitachi L6200 liquid chromatograph (Tokyo, Japan) equipped with a diode array detector UV–vis Shimadzu SPD-M6A (Kyoto, Japan) and an auto-injector (Gilson International, model 234, Barcelona, Spain). Chromatograms were recorded and processed on a LC workstation class M10A Shimadzu PC-based chromatography data system. A 20 μL sample was analyzed on a Lichrocart RP-18 reversed-phase column (250 × 4 mm, particle size 5 μm) with a precolumn C_{18} (Lichrocart 4-4, Lichrospher 100 RP-18 (5 μm)) from Merck KGaA (Darmstadt, Germany) by using mobile phase 5% formic acid (v/v) (solvent A) and HPLC grade methanol (solvent B). The flow rate was set at 1 $mL \cdot min^{-1}$. The gradient started with 20% B, increasing to 30% B at 10 min, isocratic elution at 30% B for 5 min, and increasing to 40% B at 25 min. Chromatograms were collected at 280 and 360 nm, to detect flavanones and flavones, respectively. The different phenolics were characterized by chromatographic comparison with analytical standards and quantified by absorbance of their corresponding peaks in the chromatograms. Flavanones were quantified as hesperidin, and flavones were quantified as diosmin.

Quality Parameters of Fino Lemon Juice. Titratable acidity (TA), pH, and total soluble solids (TSS) were evaluated as quality indexes. The TA was determined by titrating 2 mL of the mixture (rising to 60 mL final volume with Milli-Q water) with 0.1 N NaOH at a pH of 8.1. Results were expressed as grams of citric acid per 100 mL of sample, in accordance with AOAC (*26*). The pH values were measured at 20 °C by using a pH-meter (GLP 21, Crison Ltd., Barcelona, Spain), and TSS were recorded in a refractometer (Abbe WYA-S, Optic Ivymen System, Barcelona, Spain) at 20 °C with values expressed as °Brix.

Extraction and Analysis of Vitamin C. AA and DHAA contents were determined as described by Zapata and Dufour (*27*) with some modifications (*28, 29*). Briefly, the juice samples were centrifugated at 10500*g* (model Sigma 1-13, B. Braun Biotech International, Osterode, Germany) for 5 min at 4 °C. The supernatant was filtered through a 0.45 μm filter (Millex HV13, Millipore, Bedford, MA), and phenolic compounds in this aqueous solution were absorbed onto a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). Then, 250 μL of OPDA solution (18.8 mM) was added to 750 μL of extract for DHAA derivatization into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one. After 37 min in the dark, the samples were analyzed by HPLC. AA and DHAA contents were evaluated by using a HPLC system (Merck-Hitachi, Tokyo, Japan) equipped with an isocratic L-6000 pump, an injection valve, and a 20 μL sample loop (Rheodyne, Rohnert Park, CA) coupled to a L-4000 UV detector. Samples were analyzed on a Lichrospher 100 RP-18 reversed-phase column (250 × 4 mm, particle size 5 μm) (Teknokroma, Barcelona, Spain) with a C_{18} precolumn (Teknokroma, Barcelona, Spain). The mobile phase was MeOH/H_2O (5:95, v/v), 5 mM cetrimide, and 50 mM KH_2PO_4 (pH = 4.59). The flow rate was kept at 0.9 $mL \cdot min^{-1}$. The detector wavelength was initially set at 348 nm, and after DFQ eluted, it was manually shifted to 261 nm for AA detection. AA and DHAA were identified and quantified by comparison with pattern areas from AA and DHAA. The vitamin C content was calculated by adding AA and DHAA contents, and results were expressed as milligrams per 100 mL.

TEAC, DPPH, ABTS, and FRAP Assays of Antioxidant Capacity. All samples were centrifuged at 10500*g* (Sigma 1-13, B. Braun Biotech International, Osterode, Germany) for 5 min at 4 °C. The free radical scavenging activity was determined by using the free radical DPPH• (Sigma, Steinheim, Germany) according to Brand-Williams et al. (*30*) with some modifications (*31, 32*). The ABTS and ferric reducing antioxidant power (FRAP) methods in aqueous media were also analyzed. The antioxidant activity was evaluated by measuring the variation in absorbance at 515 nm after 50 min of reaction (DPPH), at 414 nm and 50 min (ABTS), and finally at 593 nm and 40 min (FRAP)

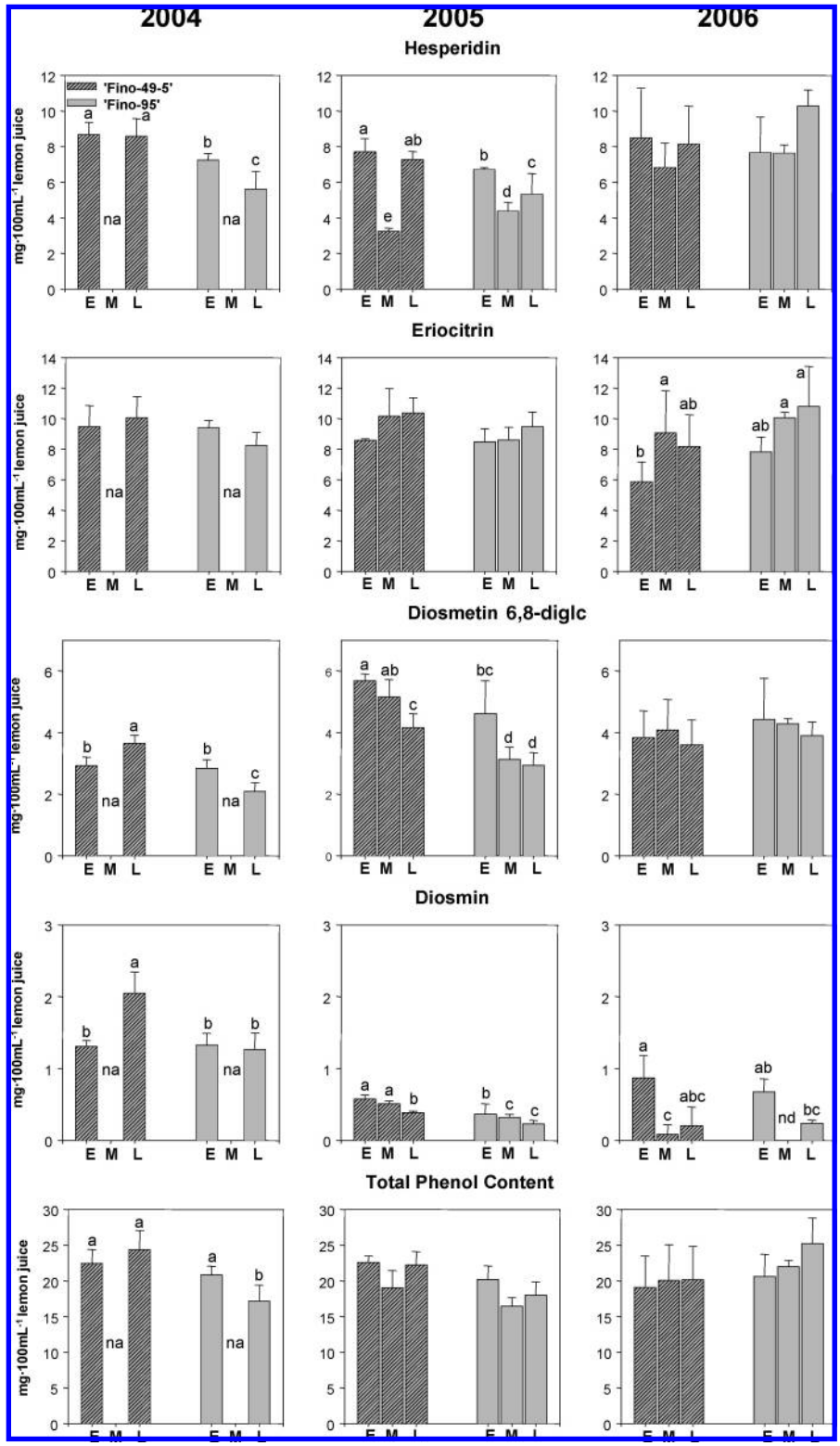

Figure 1. Individual flavonoids and total phenolic contents expressed as the sum of individual flavonoids measured by HPLC from lemon juice of Fino-49-5 and Fino-95 clones through the experimental period (2004–2006). Values are mean ($n = 4$ lemon trees) expressed as mg · 100 mL $^{-1}$ lemon juice. Means followed by different letters are significantly different (ANOVA) at $P < 0.05$ according to Duncan's test. na, not available; E, early sampling (November); M, medium sampling (February); L, late sampling (March); and nd, not detected.

(*32*). All reactions started by adding 5 μL of the corresponding diluted sample and 45 μL of MeOH or H_2O to the cuvette containing the stock solution (950 μL). The final volume of the assay was 1 mL. Reaction was followed with a spectrophotometer (UV-1603 Shimadzu, Tokyo, Japan). Results were expressed as mM trolox of equivalent antioxidant capacity (TEAC).

Statistical Analysis. All data were subjected to analyses of variance (ANOVA) by using SPSS 14.0 software (Chicago, IL). The data shown are mean values ($n = 4$), and the differences between clones and samplings were compared by using a multiple range test (least significance difference, LSD) at a $P < 0.05$ probability level (Duncan's test).

Table 3. Vitamin C (AA, DHAA, and Total Vitamin C Content) of Fino-49-5 and Fino-95 Lemon Juices through the Experimental Period (2004–2006)[a]

	Fino-49-5			Fino-95			
	E	M	L	E	M	L	LSD ($n = 4$)
				2004			
AA	33.24 a	na	35.39 a	26.10 b	na	28.35 b	(4.61)**
DHAA	7.53 a	na	2.80 b	2.86 b	na	3.84 b	(2.08)**
vitamin C	40.77 a	na	38.19 a	28.96 b	na	32.19 b	(5.09**
				2005			
AA	32.58 a	26.06 b	24.64 b	31.83 a	23.75b	25.78 b	(4.24)**
DHAA	3.82 a	2.96 bc	2.57 bc	3.28 ab	2.30c	3.35 ab	(0.76)**
vitamin C	36.40 a	29.02 b	27.21 b	35.11 a	26.05b	29.13 b	(4.62)**
				2006			
AA	24.56 a	21.05 bc	20.21 c	22.52 b	21.24bc	20.28 c	(1.86)**
DHAA	2.91 a	2.61 a	1.35 b	3.19 a	3.25a	1.60 b	(0.88)**
vitamin C	27.47 a	23.66b c	21.56 c	25.71 ab	24.49b	21.88 c	(2.27)***

[a] Values are expressed as mg·100mL^{-1} lemon juice. Means ($n = 4$) in the same row followed by different letters are significantly different at $P < 0.05$ according to Duncan's test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and na, not available.

Table 4. Antioxidant Activity Assays for the Lemon Juices Obtained from Fino-49-5 and Fino-95 Clones through the Experimental Period (2004–2006)[a]

	Fino-49-5			Fino-95			
	E	M	L	E	M	L	LSD ($n = 4$)
				2004			
ABTS	2.90 a	na	2.99 a	2.96 a	na	2.33 b	(0.33)**
DPPH	5.09 a	na	5.03 a	4.99 a	na	4.27 b	(0.54)*
FRAP	4.17 a	na	4.49 a	4.55 a	na	3.71 b	(0.39)**
				2005			
ABTS	3.29 a	3.25 a	2.75 ab	3.43 a	2.38 b	2.72 ab	(0.70)*
DPPH	3.01 a	3.32 a	2.36 b	2.87 a	3.21 a	2.35 b	(0.45)**
FRAP	3.41	2.88	2.76	3.02	2.82	2.63	(0.60)ns
				2006			
ABTS	3.98	3.40	3.80	4.02	3.49	4.15	(0.59)ns
DPPH	3.82 a	3.69 a	2.94 b	3.96 a	3.95 a	2.99 b	(0.29)***
FRAP	4.65	4.15	4.21	4.60	4.12	4.25	(0.45)ns

[a] Values are expressed as mM trolox. Means ($n = 4$) in the same row followed by different letters are significantly different at $P < 0.05$ according to Duncan's test. ns, non-significant at $P < 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; and na, not available.

RESULTS AND DISCUSSION

Environmental Parameters. The monthly averaged meteorological conditions throughout the 2004–2006 period of this study are presented in **Table 1**. The values of air temperature and relative humidity at the site were very similar through the samplings and seasons. Despite the sampling date, the averaged air temperature ranged from 12 to 16 °C, and the relative humidity of the air was always higher than 50% (**Table 1**). On the other hand, the solar radiation that the orchards received increased very similarly over the samplings, from E to L each year, increasing from 110.7 to 203.8 W·m^{-2}, respectively. The lemon tree orchards experienced sporadic short periods of rainfall without a defined pattern, varying from 1.3 to 41.3 mm/month (characteristic of a Mediterranean subtropical semiarid climate). Thus, because the fluctuations in these parameters were minimal, or in the case of rainfall, data did not support a separate discussion according to years or seasons, the climatic factors in this study may be considered quite unchanged over the time of study.

Influence of Genotype on Bioactives. The analysis of quality indexes in lemon juices over the three consecutive years (2004–2006) and corresponding sampling periods revealed analogous tendencies for the majority of the studied parameters. This is why, for clarity purposes in this section, results are presented as averaged values for the Fino lemon clones and the sampling periods.

TSS. The flavor and palatability of citrus fruit is a function of relative levels of TSS, organic acids, and the presence or absence of various aromatic or bitter juice constituents (*33*). TSS tended to decrease through the samplings (**Table 2**), and differences between clones were also detected for TSS, which was higher in Fino-49-5 than in Fino-95. Our results were similar to previous works, where values ranged from 8 to 9.5 °Brix (*14, 20*). Soluble solids concentration in lemon juice should not be ignored as quality index or parameter, although fruit quality standards for lemons do not include a minimum requirement for this parameter.

pH. Data showed significant differences between clones and samplings (**Table 2**) with pH values averaging at 2.40 ± 0.14. The pH values represented a quite conservative characteristic of the Fino clones.

Titratable Acidity. Titrable Acidity (TA) is used as an indicator of the quality of citrus juice and is also useful for determining the right harvest time for production practices (*22*). In this study, the TA values of Fino lemon juices were affected by sampling (**Table 2**), with decreasing values from E to L (15% for Fino-49-5 and 20% for Fino-95). The TA values were in the range of previously reported data (*34*).

Juice Content. According to DOCE (*35*) and MAPA (*36*), the physiological status of maturity in lemon fruits is reached once the juice content is larger than 30% of the fruit weight. We found significant differences in lemon juice contents ($P < 0.001$) between clones and seasons, with values around 25−33% (w:w) (**Table 2**). The maturity stage of the fruits, as measured by fruit yield, performed better from M to L. The juice content was relatively lower in Fino-49-5 than in Fino-95. In any case, by averaging over the 2004–2006 data, our values were lower than those found in Arabian cultivars (40% juice content) (*37*) or grafted Fino trees on different rootstocks (35–40%) (*14*).

Juice Flavonoids. Four main flavonoid compounds were detected in lemon juices (detected at 280 and 360 nm wavelengths in HPLC-DAD) with decreasing contents as follows: eriocitrin > hesperidin > diosmetin 6,8-diglucoside(diosmetin 6,8-diglc) > diosmin (**Figure 1**). Both clones presented flavanones (hesperidin diosmetin glycosides) predominating over flavones (diosmetin 6,8-diglc and diosmin) in a 3- to 4-fold ratio, as previously found in other studies (*34, 38*). Thus, eriocitrin, hesperidin, diosmetin glycosides constituted approximately 95%

Table 5. Correlation Coefficients between Phytochemicals and Antioxidant Activity Assays for Fino-49-5 and Fino-95 Clones in the Three Years Studied[a]

	2004 ($n = 8$)			2005 ($n = 12$)			2006 ($n = 12$)		
	ABTS	DPPH	FRAP	ABTS	DPPH	FRAP	ABTS	DPPH	FRAP
				Fino-49-5					
DHAA								0.714**	
AA									0.601**
vitamin C								0.529**	0.558**
hesperidin	0.537*								
eriocitrin									
diosmetin 6,8-diglc			0.557*		0.526**				
diosmin					0.584**				
total P-C									
				Fino-95					
DHAA	0.632*	0.837**	0.764**					0.717**	
AA									0.581**
citamin C								0.712**	
hesperidin	0.843**	0.752**	0.753**	0.514**					
eriocitrin	0.697**	0.607*	0.634*						
diosmetin 6,8-diglc	0.850**	0.630*	0.707**						
diosmin									
total P-C[b]	0.773**	0.694*	0.740**	0.560**					

[a] Confidence level $P < 0.05$. * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$. [b] Total phenol content (addition of the individual flavonoids).

of the total flavonic content present in Fino lemon juices, which is in agreement with Marín et al. (*34*).

Observation of the different trends of individual flavonoids revealed that the hesperidin content in 2004 samplings for Fino-49-5 remained constant, whereas it decreased by 23% in Fino-95. In 2005, hesperidin showed a significant decrease at M, probably as a result of a decreased synthesis (*39*). In 2006, there were high variations between analyses for hesperidin with no significant difference.

Eriocitrin contents in 2004 and 2005 seasons were similar in all the analyses without significant differences ($P < 0.5$) (**Figure 1**), averaging at 9.42 mg·100 mL^{-1} for both clones. The amounts of eriocitrin were similar to the range of concentrations found in Sicilian cultivars (Femminello commune, 10.5–18.8 mg·100 mL^{-1}; Interdonato, 8.4–13.9 mg·100 mL^{-1}; and Monachello, 19.0–29.8 mg·100 mL^{-1}) (*20, 40*). However, in 2006, the contents of eriocitrin increased significantly with the samplings.

Regarding the diosmetin glycosides contents in 2004, we observed higher values by the end of the harvesting season for Fino-49-5 but not for Fino-95 (**Figure 1**). In 2005, diosmetin and diosmin decreased through the successive samplings with losses ranging from 30 to 35% for both clones, and higher values were found for Fino-49-5 than for Fino-95 ($P < 0.001$). In the last year, 2006, results showed contents of diosmetin 6,8-diglc practically unaltered, but the diosmin contents varied between clones in a way similar to that observed in 2005. The highest contents of diosmin were found in the slightly immature fruits (in E, when fruits had the lowest juice content; (**Table 2**), also agreeing with Marín et al. (*41*)).

According to Del Río et al. (*8*), the decreased hesperidin contents in Fino lemons over the samplings could be explained by the inactivation of key enzymes in their biosynthetic pathways (i.e., 4′-*O*-methyltransferase), whereas the activated enzymes, such as glucosyl transferases, may produce eriocitrin, the only flavonoid that remained almost unchanged, which could be synthesized earlier in the pathway. On the other hand, Del Río et al. (*8*) reported contents of diosmin twice higher than those of individual flavanones, a pattern that is different from the one observed in our clones and in other reports (*20*), in which the diosmin contents were the lowest. Regarding the individual flavonoids, our results were similar to those found in Sicilian cultivars (*20*) (i.e., Interdonato lemon tree), characterized by a specific flavonoid pattern with an eriocitrin/hesperidin ratio of 1:1 (**Figure 1**). So, the observed pattern of flavonoids may allow us to perform an easy phytochemical characterization of the cultivars/clones.

The total flavonoid content determined by HPLC-DAD analysis was expressed as total content in **Figure 1** and was determined by the addition of the contents of the individual flavonoids showing significant differences ($P < 0.001$) in 2004 (**Figure 1**), with values ranging from 16 to 26 mg·100 mL^{-1} of total flavonoids (phenolics) in Fino lemon juice.

Vitamin C Content. The vitamin C components (AA, DHAA, and total vitamin C as AA+DHAA) presented values in agreement with values from previous authors, where AA was the predominant component of total vitamin C in lemon juice (*42*). Changes in vitamin C through the sampling periods are shown in **Table 3**, and higher contents of AA and vitamin C were found through the samplings for Fino-49-5 and Fino-95 ($P < 0.01$). We detected a significant decrease (60%) of the DHAA content in Fino-49-5 through the samplings ($P < 0.01$). Initial E values were higher than those in M and L periods for each component (DHAA ($P < 0.01$), AA ($P < 0.01$), and vitamin C ($P < 0.001$)). The influence of weather conditions (air temperature in the orchards, foliage of the trees shading the fruits, hours and quality of solar radiation, etc.) may play a more important role in partly explaining the decreased vitamin C contents as found through the sampling seasons (*42*) over the three years of study than the selected clones do. In any case, the averaged values of the registered environmental data (**Table 1**) were not enough to explain the analytical results, and increased juice content and solar radiation, for example, could also be related to the increased biomass and fruit growth, with the possibility of a dilution effect in terms of vitamin C content, a possibility that needs to be further investigated. It would be interesting to explore why we could not find any transformation from AA to DHAA (data not shown), and why the analyzed samples suffered significant losses because of degradation. For practical applications, the vitamin C content in Fino-49-5 and Fino-95 clones from southeastern Spain ranged from 25 to 41 mg·100 mL^{-1} in prepared juices.

Antioxidant Capacity. Antioxidant capacity of the Fino lemon juices was evaluated by the DPPH, ABTS, and FRAP assays (**Table 4**). In 2004, there was no remarkable value of antioxidant capacity in any of the studied clones or harvests,

and the lowest values were found in L for Fino-95. In 2005, ABTS, DPPH, and FRAP tests revealed values around 3 mM TEAC and lower activity by the end of the sampling seasons (**Table 3**). In general, results were very similar to those of 2004. Again, in 2006, values from ABTS, DPPH, and FRAP were quite similar between cultivars and samplings, despite the observed decrease in antioxidant capacity by the end of each sampling season ($P < 0.001$) (**Table 4**).

Correlations between Antioxidant Capacity and Bioactives. Table 5 shows a high variability in the analysis of the correlation between lemon juice bioactives and the tested in vitro antioxidant activity. In this sense, it is noticeable that Fino-49-5 presented only two correlation factors higher than 0.6 ($R^2 > 0.7$ DPPH−DHAA and $R^2 > 0.6$ FRAP−AA in the 2006 season). On the other hand, Fino-95 presented many factors of correlation in the 2004 season data (between the three assayed tests of antioxidant activity and the major bioactive compounds (**Table 5**)), whereas in the 2005 and 2006 seasons, we only determined two R^2 values higher than 0.6 (**Table 5**). So, it was difficult to establish a clear relationship between the antioxidant capacity and bioactive compounds of lemon juice, maybe as a result of a limited number of samplings in this three-year study.

In summary, the nutritive value of the different lemon juices obtained was very consistent and quite similar among clones, samplings, and seasons. Thus, although Fino-49-5 lemons performed better in terms of vitamin C and phenolic content in juice, the range of variation between the clones through the three consecutive years of study does not exclude any of them for industrial food processing. Further research to investigate the effect of the environmental parameters, such as irrigation, fertilization, temperature stress, and so forth, would help explain the sources of variations found here. The environmental conditions were probably not determinant factors on the bioactive content of the lemon juice. The lemon juice flavonoids may allow us to easily characterize the type and/or cultivars/clones (i.e., chemical markers). Therefore, the food industry would have phytochemically rich and nutritive lemons produced in Murcia (southeastern Spain), with practically complete independence of the harvesting season and choice of clone.

ACKNOWLEDGMENT

We appreciate the assistance of Dr. Ma. Isabel Sánchez Toribio with the information of the weather parameters at La Matanza (Santomera, Murcia).

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Received for review November 9, 2007. Accepted January 4, 2008. This study is part of the research project of E.G.-M.'s Ph.D. thesis under the CSIC-I3P Fellowship Programme, partially funded by the European Social Fund (ESF). The authors wish to thank the Fundación Séneca and the Murcia Regional Government for financial support through the Research Project AGR/12/FS/02. D.A.M. thanks the Spanish Ministerio de Educación y Ciencia, CSIC, and the European Social Fund for funding through the Ramón y Cajal S&T Programme.

JF073282W