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Main Organic Acid Distribution of Authentic Citrus Juices in Turkey

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Abstract: Nineteen authentic citrus juice samples (6 tangerine, 4 sweet orange, 4 grapefruit, 4 lemon and 1 sour orange) were investigated with respect to organic acid distribution. Average citric, malic and fumaric acid contents of tangerine juices were 9.22 g l^{-1}, 5.29 g l^{-1} and 368 µg l^{-1}, respectively. These values for orange juices were 13.28 g l^{-1}, 7.79 g l^{-1} and 373 µg l^{-1}, respectively. The citric acid concentration in lemon juices, having the highest acidity, varied between 48.54 and 60.32 g l^{-1}. It was followed by grapefruit juices with an average 19.61 g l^{-1} citric acid content. In all citrus juices, the most abundant organic acid was citric acid, ranging from 6.05 to 60.32 g l^{-1}, with the exception of one tangerine variety. Malic acid was the second most abundant acid (1.27-12.15 g l^{-1}), followed by fumaric acid (0-807 µg l^{-1}). Grapefruit, lemon and sour orange juices did not contain fumaric acid.

Key Words: organic acid, orange, grapefruit, lemon, tangerine

Türkiye'deki Doğal Turunçgil Sularında Başlıca Organik Asitlerin Dağılımı

Özet: 19 adet doğal turunçgil meyve suyu örneğinde (6 mandarin, 4 portakal, 4 greyfrut, 4 limon ve 1 turunç) organik asit dağılımı araştırılmıştır. Mandarin sularındaki sitrik, malik ve fumarik asit miktarları ortalama olarak sırasıyla 9.22 g l^{-1}, 5.29 g l^{-1} ve 368 µg l^{-1} olarak belirlenmiştir. Portakal sularında ise bu asitlere ait değerler sırasıyla 13.28 g l^{-1}, 7.79 g l^{-1} ve 373 µg l^{-1} olarak saptanmıştır. Asitliği en yüksek olan limon sularında sitrik asit miktarı 48.54-60.32 g l^{-1} arasında değişim göstermektedir. Bunu ortalama 19.61 g l^{-1} sitrik asit miktarı ile greyfrut suyu izlemektedir. Mandarin varyetelerinden bir tanesi hariç, turunçgil sularındaki başat organik asit 6.05 – 60.32 g l^{-1} arasında değişen sitrik asittir. Turunçgil sularında yaygın olarak bulunan ikinci asit malik asittir (1.27-12.15 g l^{-1}) ve bunu fumarik asit (0-807 µg l^{-1}) izlemektedir. Greyfrut, limon ve turunç suları fumarik asit içermemektedir.

Anahtar Sözcükler: organik asit, turunçgil, portakal, greyfrut, limon, mandarin

Introduction

Organic acids are a useful index of authenticity in fruit products, since they have lower susceptibility to change during processing and storage than other components of fruits (Camara et al., 1994). Accurate knowledge of organic acid levels (and ratios) might be useful for determining the percentage juice content of juice products, and also for detecting misbranding and/or adulteration in this food class (Coppola and Starr, 1986), since each fruit has a unique pattern of organic acids (Wrolstad, 1981). The organic acid composition of fruits is also of interest because of its important influence on the sensory properties of fruits and fruit juices. Even though they are minor components of fruits, in combination with sugars, they are important attributes of the sensory quality of raw and processed fruits (Wang et al., 1993). At the same time, some organic acids may be used as indicators of ripeness (Palmer and List, 1973), bacterial activity and adulteration (Evans et al., 1983; Blanco et al., 1996).

Citrus fruits are classified as acid fruits, since their soluble solids are composed mainly of organic acids and sugars (Kale and Adsule, 1995), which are used as the main index of maturity and one of the major analytical measures of flavor quality (Fellers, 1991). The main acids of citrus fruits are citric and malic acids. In addition, traces of tartaric, benzoic, oxalic and succinic acids have also been reported (Kale and Adsule, 1995).

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The present study was undertaken to determine the organic acid distribution of citrus juices prepared from different species and varieties grown in Turkey.

Materials and Methods

Materials

All citrus varieties were taken from the Mediterranean region, which is the main citrus growing area in Turkey. Fruits were supplied by the Citrus Fruits and Greenhouse Research Center located in Antalya. All the samples were stored at +5 °C and processed into fruit juice within 1 week.

i. Fruit Juice Processing: Fruits were washed in tap water and then cut in half. Fruit juice was extracted using a lab scale citrus juice extractor (Moulinex T574, France). After juice extraction, raw juice was heated at 96 °C for 1 min to inactivate enzymes. Following the heating process, the juices rapidly cooled down to room temperature, were filtered through 8-folded cheesecloth to eliminate particulates and then put into 200 ml glass bottles. After bottling, all juice samples were pasteurized at 96 °C for 20 min. Then, the samples were cooled with tap water and stored at -18 °C until analyzed.

Methods

The pH value of citrus juices was determined with a pH meter (CONSORT P 407, SCHOTT GERATE, Belgium). Titratable acidity (TA) was measured by direct titration of a 10 ml sample with 0.1 N NaOH to pH 8.1 and the results were expressed as citric acid. A refractometer (N.O.W., Nippon Optical Work Co., Ltd., Tokyo, Japan) was used to determine the soluble solids content of juices.

i. Organic acid analysis: A slightly modified HPLC method (Evans et al., 1983) was used to determine the organic acid profile of citrus juices. The peaks were identified by a comparison of the retention time of standard acids and spiking the sample with standard compounds.

ii. Sample preparation: After dilution, juice samples were centrifuged at 4000 rpm for 2 min. Supernatant was filtered with filter paper before being passed through a 0.45 µ Millipore membrane filter (HAWP Millipore Co., Bedford, MA, USA). Then 20 µl samples were injected into the HPLC.

iii. Organic acid standards: Organic acids (citric, fumaric, malic) were obtained from Aldrich Co. (Sigma-Aldrich Chemie, Steinheim, Germany). The standard solutions were prepared individually at different concentrations with double distilled water.

iv. HPLC conditions: Analyses were carried out on a Bio Rad Aminex HPX-87 ion exclusion column (300 x 7.8 mm) preceded by a Cation-H Bio Rad Micro-Guard column (30 x 4.6 mm) installed in a Waters liquid chromatograph equipped with a Waters 510 pump and a reodyne injector. Column effluents were monitored by a UV-VIS detector (Waters 486) set at 214 nm. Integration and data storage were performed by Millennium 2010 Chromatography software (Millipore Co., Milford, MA, USA). The mobile phase (0.01 N H_2SO_4), used at a flow rate of 0.6 ml/min, was filtered through a 0.45 µ Millipore membranefilter (HAWP Millipore Co.) and degassed in a vacuum.

v. Statistical analysis: Experiments were replicated twice and all analyses were performed in duplicate. The significance of differences among different species and different varieties was determined at the 95% level of Tukey's family error rates, using the Minitab Statistical software (Minitab for Windows, version 13, Minitab, Inc. 1998).

Results and Discussion

A representative chromatogram of organic acids in citrus juices analyzed by HPLC is shown in Figure 1. The peaks at 8.42, 10.08 and 16.57 min represent citric, malic and fumaric acids, respectively.

The parameters of the major organic acids, titratable acidity, pH and total soluble solids in citrus juices are given in Table 1. As indicated by other researchers (Clements, 1964; Yamaki, 1989), citric acid is the main organic acid found in citrus juices (6.05-60.32 g l^{-1}), except for Clementine tangerine, which contains 12.15 g l^{-1} malic acid and 6.05 g l^{-1} citric acid. The second most abundant acid was malic acid (1.27–12.15 g l^{-1}) in all the citrus juices analyzed except for Clementine. Fumaric acid, not detected in grapefruit, lemon or sour orange juices, was 81-807 µg l^{-1} in the other citrus juices. Lee (1993) also reported 0.4 mg 100 ml^{-1} fumaric acid in orange juice. He also found traces of oxalic, quinic, tartaric, isocitric, ascorbic and cis-aconitic acids. In a study of 42 citrus species, smaller amounts of acetate,

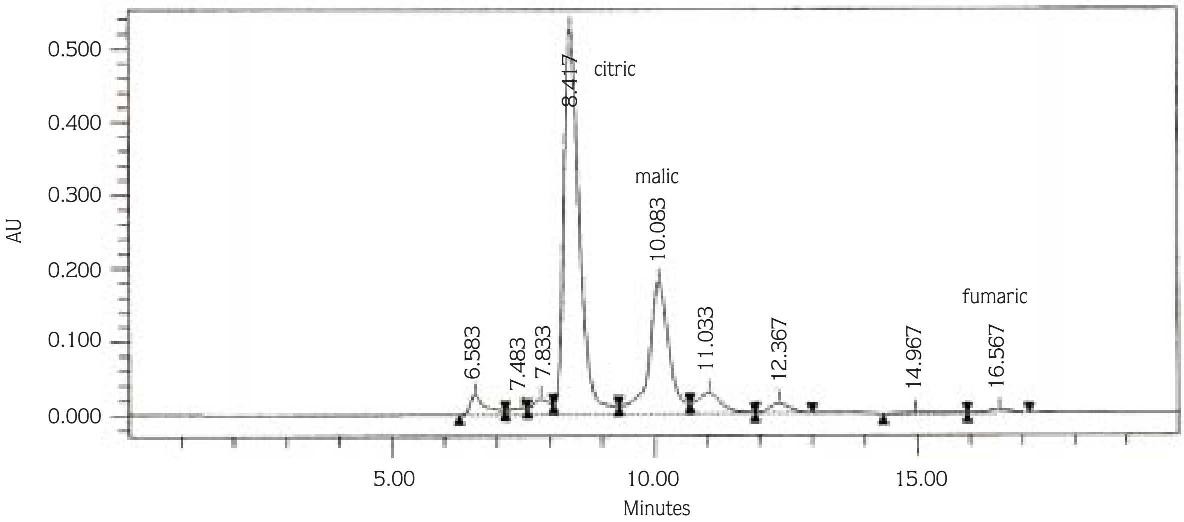

Figure 1. An HPLC chromatogram of organic acids in authentic orange juices.

Table 1. Organic acid contents of authentic citrus juices.

Species	Variety	Citric (g l^{-1})	Malic (g l^{-1})	Fumaric (µg l^{-1})	T Acid (g l^{-1})	pH	TSS, %
Tangerine (*Citrus reticulata*)	Fremond	6.70 ± 0.021	5.90 ± 0.141	99 ± 28.3	8.13 ± 0.269	3.47 ± 0.028	12.0 ± 0.283
	Authentic	12.04 ± 0.156	4.32 ± 0.056	626 ± 7.07	12.10 ± 0.092	3.44 ±0.017	10.7 ± 0.354
	Fairchild	9.22 ± 0.028	5.44 ± 0.077	442 ± 8.49	10.24 ± 0.085	3.51 ± 0.021	13.1 ± 0.317
	Clementine	6.05 ± 0.049	12.15 ± 0.085	504 ± 1.41	8.07 ± 0.361	3.69 ± 0.020	12.1 ± 0.283
	Minneola	12.14 ± 0.205	1.27 ± 0.091	251 ± 7.07	13.95 ± 0.184	3.17 ± 0 014	10.8 ± 0.283
	Satsuma	9.14 ± 0.156	2.63 ± 0.113	289 ± 7.85	10.03 ± 0.173	3.46 ± 0.014	8.4 ± 0.141
	Minimum	6.05	1.27	99	8.07	3.17	8.4
	Maximum	12.14	12.15	626	13.95	3.69	13.1
	Average	9.22	5.29	368	10.42	3.46	11.2
Sweet orange (*Citrus sinensis*)	Washington	11.10 ± 0.021	9.13 ± 0.127	87 ± 5.66	11.14 ± 0.092	3.48 ± 0.014	11.5 ± 0.283
	Valencia	14.44 ± 0.099	8.52 ± 0.219	81 ± 2.83	14.50 ± 0.134	3.34 ± 0.014	14.0 ± 0.141
	Shamouti	11.94 ± 0.219	4.84 ± 0.191	517 ± 27.6	12.29 ± 0.092	3.34 ± 0.021	11.4 ± 0.283
	Authentic	15.65 ± 0.057	8.67 ± 0.134	807 ± 62.2	15.57 ± 0.085	3.22 ± 0.021	11.5 ± 0.283
	Minimum	11.10	4.84	81	11.14	3.22	11.4
	Maximum	15.65	9.13	807	15.57	3.48	14.0
	Average	13.28	7.79	373	13.38	3.34	12.1
Grapefruit (*Citrus paradisi*)	Henderson	19.36 ± 0.184	4.80 ± 0.042	n.d.	21.39 ± 0.078	3.00 ± 0.007	10.1 ± 0.424
	Marsh Seedless	24.25 ± 0.474	3.48 ± 0.170	n.d.	25.12 ± 0.453	3.04 ± 0.007	11.0 ± 0.283
	Red Blush	17.87 ± 0.361	3.44 ± 0.106	n.d.	18.88 ± 0.156	2.99 ± 0.014	9.5 ± 0.424
	Star Ruby	16.96 ± 0.106	4.40 ± 0.021	n.d.	17.23 ± 0.078	2.99 ± 0.007	10.5 ± 0.287
	Minimum	16.96	3.44	-	17.23	2.99	9.5
	Maximum	24.25	4.80	-	25.12	3.04	11.0
	Average	19.61	4.03	-	20.65	3.00	10.3
Lemon (*Citrus limon*)	Kütdiken	60.32 ± 0.311	4.97 ± 0.042	n.d.	63.68 ± 0.453	2.41 ± 0.007	7.4 ± 0.212
	Karalimon	48.54 ± 0.042	7.13 ± 0.028	n.d.	52.80 ± 0.453	2.46 ± 0.007	8.5 ± 0.283
	Interdonato	51.87 ± 0.431	5.07 ± 0.049	n.d.	57.20 ± 0.792	2.42 ± 0.014	6.6 ± 0.141
	Lamas	59.72 ± 1.216	6.82 ± 0.099	n.d.	62.08 ± 0.453	2.44 ± 0.014	9.6 ± 0.354
	Minimum	48.54	4.97	-	52.8	2.41	6.6
	Maximum	60.32	7.13	-	63.68	2.46	9.6
	Average	55.11	6.00	-	58.94	2.43	8.0
Sour orange (*Citrus aurantium*)		48.79 ± 0.431	2.21 ± 0.156	n.d.	48.48 ± 0.679	2.60 ± 0.021	10.0 ± 0.283

T. Acid: titratable acidity, TSS: total soluble solids, n.d.: not detected pyruvate, oxalate, glutarate, fumarate, formate, succinate and α-ketoglutarate were also reported by Yamaki (1989). Ascorbic acid, which ranged between 35 mg 100 ml^{-1} and 55 mg 100 ml^{-1} in citrus fruits, could not be determined in this research. This might be due to the analytical conditions used, since ascorbic acid in fruits and vegetables was mainly quantified by RP-18 column.

Average titratable acidities in sweet orange and grapefruit juices were 13.38 g l^{-1} and 20.65 g l^{-1}, respectively, while these results were 12.2 and 17.5 g l^{-1}, respectively, in Israeli orange and grapefruit juices (Cohen, 1988). Average malic acid was the highest in sweet orange juices (7.79 g l^{-1}), followed by lemon (6.00 g l^{-1}), tangerine (5.29 g l^{-1}), grapefruit (4.03 g l^{-1}) and sour orange juices (2.21 g l^{-1}). Citric acid values of sweet orange and grapefruit juices were close to or within the range given by Gherardi et al.(1995), AIJN (1993), and Walrauch and Faethe (1988), while malic acid contents were higher than the literature values reported by Cohen (1988), and Cunha et al.(2002). The titratable acidity of lemon juices (52.8-63.68 g l^{-1}) was similar to Sinclair's (1984) results, which varied between 62.8 and 64.4 g l^{-1}.

The effect of species on the organic acid distribution of citrus fruit juices is shown in Table 2. Tangerine and sweet orange juices, which have the lowest acidity, show no differences in respect of organic acids, pH, titratable acidity or total soluble solids. Grapefruit juice is more acidic (20.65 g l^{-1}) than tangerine and sweet orange juices and citric acid contents of grapefruit juices were significantly different ($P < 0.05$) than that of fruit juices of other species. The highest acid content was found in lemon juices (average 58.94 g l^{-1}). Baldwin (1993) also reported that tangerines and oranges have the lowest acidity, while lemons, limes and sour oranges have the highest acidity among citrus fruits. The malic acid content of sweet orange juices was significantly different ($P < 0.05$) from that of sour orange and grapefruit juices. Grapefruit, sour orange and lemon juices were significantly different ($P < 0.05$) from other samples (except for sour orange juice) with respect to fumaric acid.

Conclusion

The determination of organic acids in citrus fruits showed that citric acid was the main acid in all citrus juices except for one of tangerine variety (Clementine). Malic acid was the second most abundant acid in citrus juices. Grapefruit, lemon and sour orange species did not contain fumaric acid. In addition, these species were significantly different from tangerine and sweet orange species with respect to higher titratable acidity.

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Table 2. Effect of species on organic acid distribution of authentic citrus juices[a].

Species	Citric (g l^{-1})	Malic (g l^{-1})	Fumaric (µg l^{-1})	T.acid (g l^{-1})	pH	TSS, %
Tangerine	9.22a	5.29ab	368.4a	10.42a	3.46a	11.2ab
Sweet orange	13.28a	7.79a	373.0a	13.38a	3.34a	12.1a
Sour orange	48.79b	2.21b	0.0ab	48.48b	2.60b	10.0abc
Grapefruit	19.61c	4.03b	0.0b	20.65c	3.00c	10.3b
Lemon	55.11b	6.00ab	0.0b	58.94d	2.43b	8.0c

T. Acid: titratable acidity, TSS: total soluble solids

[a] mean values in the same column not sharing superscript lower case letters are significantly different ($P < 0.05$)

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