CHEMICAL COMPOSITON OF PEACHES USED FOR COMMERCIAL JUICE PRODUCTION IN TURKEY: SUGARS, ORGANIC ACIDS AND AMINO ACIDS

TÜRKİYE'DE TİCARİ MEYVE SUYU ÜRETİMİNDE KULLANILAN ŞEFTALİLERİN KİMYASAL BİLEŞİMİ: ŞEKER, ORGANİK ASİTLER VE AMİNO ASİTLER

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ABSTRACT: The chemical composition of fresh juices of peaches from Adana (12 lots) and from central (20 lots) and 4 different provincial regions of Mersin (Erdemli-13 lots, Tepeköy-12 lots, Çandır-9 lots, Kızılbağ-6 lots) as well as their commercially processed juice concentrate (in aseptic packages, 25 lots) were determined in terms of sugars, organic and amino acids. The sucrose (24.07-49.56 g/kg), fructose (13.59-28.43 g/kg) and glucose (7.20-15.26 g/kg) contents and also citric (2.26-6.26 g/kg) and malic (2.05-4.95 g/kg) acids as the main organic acids were determined simultaneously using HPLC technique by connecting refractive index and variable wavelength UV-Vis (VWD) detectors in series. Asparagine (1749.34-4043.14 mg/L together with serine) the main amino acid found in peach, aspartic acid (24.18-123.46 mg/L), glutamic acid (31.54-90.75 mg/L), serine, histidine (7.97-27.35 mg/L), threonine (7.24-88.51 mg/L), β-alanine (10.25-46.96 mg/L), γ-aminobutyric acid (30.24-130.15 mg/L), valine (36.58-223.42 mg/L), methionine (9.94-46.02 mg/L) and ornithine (3.56-30.14 mg/L) contents were also determined by HPLC using VWD. Total titratable acidity, total soluble solids and pH values of the samples were also determined. The chemical composition of peaches from different regions and their commercially processed concentrates were statistically analyzed using Tukey's classification and compared with the ranges given in AIJN (Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union) standard.

Keywords: Peach, chemical composition, sugar, organic acid, amino acid

ÖZET: Adana (12 parti) ve Mersin'in merkez (20 parti) ile 4 farklı bölgesinden (Erdemli-13 parti, Tepeköy-12 parti, Çamdır-9 parti, Kızılbağ-6 parti) gelen şeftalilerin ve ticari meyve suyu konsantresinin (aseptik dolum paketleri halinde-25 parti) kimyasal bileşimi şeker, organic asit ve amino asit cinsinden belirlenmiştir. Şekerlerden sakkaroz (24.07-49.56 g/kg), fruktoz (13.59-28.43 g/kg) ve glukoz (7.20-15.26 g/kg) içerikleri ile başlıca organik asitlerden sitrik (2.26-6.26 g/kg) ve malik (2.05-4.95 g/kg) asit miktarları HPLC tekniği kullanılarak eş zamanlı saptanmıştır. Bu amaçla, refraktif indeks (RID) ve değişken UV-Vis dalga-boyu (VWD) detektörleri seri olarak birbirine bağlanmıştır. Şeftalide en çok bulunan asparajin (1749.34-4043.14 mg/L, serin ile birlikte), aspartik asit (24.18-123.46 mg/L), glutamik asit (31.54-90.75 mg/L), serin, histidin (7.97-27.35 mg/L), treonin (7.24-88.51 mg/L), β-alanin (10.25-46.96 mg/L), γ-aminobütirik asit (30.24-130.15 mg/L), valin (36.58-223.42 mg/L), metiyonin (9.94-46.02 mg/L) ve ornitin (3.56-30.14 mg/L) miktarları da VWD kullanılarak HPLC ile saptanmıştır. Ayrıca, örneklerin toplam titre edilebilir asitlik, toplam çözünebilir katı madde miktarları ile pH değerleri de saptanmıştır. Değişik bölgelerden gelen şeftaliler ile ticari üretilmiş şeftali konsantresinin kimyasal bileşimleri Tukey'in sınıflama (Tukey's classification) yöntemi kullanılarak istatistiksel olarak değerlendirilmiş ve sonuçlar AJIN standardında (Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union- AB Meyve ve Sebzelerden elde edilen Meyve suyu ve Nektarları Endüstrisi Birliği) verilen aralıklarla karşılaştırılmıştır.

Anahtar kelimeler: Şeftali, kimyasal bileşim, şeker, organik asit, amino asit

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INTRODUCTION

Fruit juices each have very distinct organic acid and sugar profiles (1-5). Several methods for the separation and quantification of the organic acid and sugar components have been developed (6-7). Castellari and coworkers (8) developed a HPLC method for the simultaneous detection of organic acids and carbohydrates in grape musts and wines which was then adapted to fruit juices by Chinnici et al. (9).

Amino acid profile analysis and determination of key amino acids as markers have been studied for various fruits and plants (10-16). Several HPLC methods have been developed for amino acid detection using various derivatization procedures and dependently different detectors as variable wavelength, UV-Vis or fluorescence (2, 17-18).

Several studies are conducted in Turkey towards the determination of various components of fruits and their juices (19-24). Unfortunately, there is a gap in studies concerning chemical composition of fruits, especially of peaches, in terms of individual sugars, organic acids and amino acids.

Peaches grown in Turkey, both fresh and processed as juice are mostly exported with the main proportion to EU. However, one of the major problems faced is the rejection of fruit juice products due to adulteration. In fact the problem arises from that the chemical composition of the fruits processed into juice might sometimes fall outside the ranges given in some standards as AIJN (25). The purpose of our study was to partially fulfill the gap of data related to the chemical composition of the peaches grown in Turkey which might be made use by the fruit juice industry. Çukurova region with 70 % of the peach juice production capacity of Turkey was chosen as the experimental study area. In this respect, the chemical composition of peach and its commercial juice was determined in terms of individual sugars, organic acids and amino acids while adopting procedures for routine analyses of commercially important fruits grown in Turkey. The results were compared with the ranges given in AIJN standard since highest export of peach juices is practiced to European countries.

MATERIALS AND METHODS

Material

Fresh peach fruits from Adana (35:18E, 37:01N) and from central and 4 different provincial regions of Mersin (34:38E, 36:48N) (Erdemli, Tepeköy, Çandır, Kızılbağ) as well as their commercially processed juice concentrate (in aseptic packages) were supplied by ETAP Tarım AŞ. daily from 03 August to 09 September 2004 (Table 1). Except Erdemli which is at sea level, all the other provincial regions of Mersin lie above 1000 m from sea level. Commercially processed juices were random blends of peaches from different regions.

Each peach lot was analyzed for pH, total soluble solids (°Brix) and total titratable acidity at the plant site before delivering to the laboratory of the Food Engineering Department. Randomly 15 samples were selected from each fruit lot. The samples were processed by a domestic juice extractor (Arçelik K1573, Istanbul, Turkey). The mash obtained, as well as the commercial juice concentrate were then centrifuged (Hettich 1100, Germany) at 18 000 rpm for 15 min at +4°C to collect supernatant. The supernatant (juice) was then analyzed for amino acids, sugars and organic acids. All chemical analyses were carried out in duplicates.

All chemicals were either of analytical or HPLC grade and except L-tryptophane (Sigma, St. Louis, USA) were purchased from Merck (Darmstadt, Germany).

Determination of total soluble solids, total titratable acidity, pH.

Total soluble solids (RFM 340 refractometer, Bellingham), total titratable acidity in terms of citric acid and pH (model, pH-meter) were determined according to AOAC methods (26).

High performance liquid chromatography (HPLC)

The High Performance Liquid Chromatography (HPLC) system used was an Agilent 1100 Series equipped with a variable wavelength UV-Vis detector, a refractive index detector and a ChemStation (Agilent Tech. 1999-

Table 1. Number of peach lots from each region and their arrival dates

Region	August 2004																																September 2004								
	03	04	05	06	07	08	09	10	11	12	13	14	15	18	19	20	21	22	23	24	25	26	27	28	29	30	31	01	02	03	04	05	06	07	08	09
Adana	√	√	√		√			√	√		√		√√			√	√		√					√													
Mersin		√	√		√				√	√		√		√		√	√√	√	√	√	√			√		√				√		√		√	√	√
Erdemli					√			√			√	√	√		√		√	√		√	√					√						√			√√	
Tepeköy						√						√			√√				√			√	√	√			√			√				√	√		
Çandır						√	√	√		√													√	√		√			√						√		
Kızılbağ							√				√												√	√		√			√								
Aseptical Packaging										√			√				√√	√	√√	√	√√	√√	√	√√√	√		√	√√		√	√√	√	√			√

√ one lot
√√ two lots
√√√ three lots

2000) software for Liquid Chromatography. All samples were filtered through a 0.45 mm nylon filter (Millipore) before injection. Twenty microliters of filtered sample was directly injected into HPLC.

Determination of amino acids.

The individual amino acids of peach samples were determined according to Hermosin et al. (13) with slight modifications. Tryptophane was used as internal standard

Derivatization.

0.5 mL juice samples were mixed with 0.5 mL internal standard (0.1% w/v tryptophane), 30 mL diethyl ethoxymethylenemalonate, 1.5 mL ethanol and 3.5 mL borate buffer (1 M, pH 9.0) in a 10 mL tube with screw tap. The tubes were then placed in an ultrasonic bath (Elma, transonic 460/H, Germany) for 30 min at room temperature. The derivatized samples were diluted with mobile phase prior direct injection into HPLC.

Column and mobile phase for amino acid determination.

A C-18 column (250x4.6 mm id ACE 5 micron A114422) was used. UV-Vis detector was set at 280 nm. Elution solvents were: Solvent A: 25 mM acetate buffer (pH 5.8), Solvent B: pure acetonitrile. The flow rate of the mobile phase was 1.5 mL/min.

2.4.3. Analytical conditions. Linear gradient program was set as: 6% A initially, 12% A at 13.0 min, 14% A at 13.5 min, 18% A at 17 min, 22% A at 20 min, 32% A at 32 min and 6% A at 35 min. Identification and quantification was done using pure amino acids.

Determination of organic acids and carbohydrates.

Individual organic acids and carbohydrates were determined according to Castellari et al. (8). Diluted juice samples were directly injected into HPLC.

Column and mobile phase for sugar and organic acid determination.

An Aminex HPX-87H column (125-0140, 300x7.8 mm) was used. UV-Vis set at 210 nm and refractive index detectors were connected in series. As mobile phase, 0.045 N sulfuric acid containing 6% acetonitrile was used.

Analytical conditions.

The samples were isocratically separated at 0.5 mL/min flow rate.

Identification and quantification was done using pure sugars and organic acids.

Statistical Analysis

Statistical analysis of variance (ANOVA) was applied and significant ($p<0.05$) difference between means was determined using the Tukey's classification. The analyses were performed with STATISTICA statistical program version 5.0

RESULTS AND DISCUSSION

Minimum and maximum values for amino acids, sugars and organic acids content of peaches from different regions and that of commercially processed samples are given in Table 2.

Table 2. Minimum and maximum values for (a) individual sugars, organic acids, total titratable acidity, total soluble solids, pH and (b) amino acids in peach samples from different regions

(a)

Region	Sucrose (g/kg fruit juice)	Glucose (g/kg fruit juice)	Fructose (g/kg fruit juice)	Sitric acid (g/kg fruit juice)	Malic acid (g/kg fruit juice)	TTA (meq/mL)	°Brix	pH
Adana	25.92-49.56	9.28-15.26	17.64-28.43	3.07-5.45	2.45-4.49	0.50-0.72	9.33-12.47	3.57-3.79
Mersin	30.27-44.69	7.86-12.48	14.57-22.00	2.54-5.70	2.40-3.78	0.53-0.65	9.92-10.40	3.55-3.80
Erdemli	29.77-40.69	8.66-12.47	15.83-20.91	2.94-4.88	2.05-3.98	0.55-0.71	9.78-10.93	3.59-3.84
Tepeköy	29.23-51.49	7.20-13.11	13.59-23.46	2.26-4.25	2.70-4.29	0.55-0.66	10.00-10.52	3.58-3.84
Çandır	28.16-44.28	9.51-14.06	17.22-25.40	3.64-6.26	2.64-4.65	0.52-0.78	10.05-10.92	3.73-3.86
Kızılbağ	24.07-47.89	9.21-12.51	17.58-22.54	3.17-5.49	2.68-4.73	0.47-0.66	10.29-11.43	3.62-3.88
Aseptical Packaging	26.30-32.24	10.08-13.69	18.87-22.80	2.61-3.80	3.23-4.95	0.60-0.71	9.69-10.06	-
AIJN	12-60	7.5-25	10-32	1.5-5.0	2-6			

(b)

Region	Asp_Ser (mg/L)	Aspartic acid (mg/L)	Glutamic acid (mg/L)	Histidine (mg/L)	Threonine (mg/L)	Alanin (mg/L)	GABA (mg/L)	Valine (mg/L)	Methionine (mg/L)	Ornitine (mg/L)
Adana	2297.55-4043.14	24.18-60.10	31.54-77.90	7.97-18.26	13.60-25.83	15.69-27.36	35.02-67.72	36.58-101.33	11.18-28.01	6.36-20.72
Mersin	2045.27-3187.91	33.85-89.53	41.30-76.29	8.89-21.57	11.98-29.78	13.60-35.79	30.24-77.37	87.06-176.12	9.94-25.66	4.03-14.91
Erdemli	1749.34-2675.85	46.81-69.43	49.12-74.82	12.37-15.77	7.24-37.10	10.25-21.46	33.81-59.79	96.95-223.42	11.32-18.95	3.56-21.10
Tepeköy	1783.96-3028.49	34.35-85.76	58.76-78.51	11.39-21.43	12.26-40.21	14.59-23.05	39.07-73.54	86.50-169.20	12.18-27.25	6.91-21.08
Çandır	2034.74-3522.51	41.79-69.62	39.24-70.65	12.72-21.70	12.32-17.07	13.05-36.19	45.53-83.54	60.05-119.69	15.06-28.41	4.09-21.26
Kızılbağ	1752.57-3447.25	59.50-92.20	35.77-90.75	8.88-27.35	11.91-88.51	16.07-46.96	67.71-130.15	72.34-170.60	12.74-46.02	4.68-22.69
Aseptical Packaging	2080.46-2882.59	89.17-123.46	58.23-76.25	10.60-16.41	16.42-36.72	18.88-31.90	55.56-87.04	103.89-193.20	16.10-33.06	13.59-30.14
AIJN	*	50-330	15-200	≤20	10-80	40-300	5-150	5-50	5-30	≤20

* for asparagine 1500-4500, for serine 30-350 mg/L

There were no significant differences among the peach samples from different regions in terms of pH and total titratable acidity contents at the $p<0.05$ level. The Brix values of aseptically packaged samples were significantly lower from that of Adana ($p<0.05$).

The total sugar to total soluble solids ratio was around 0.65 for each region.

Sugar content of the juices of peach samples from different regions and aseptic packaging

The major sugars in peach are sucrose, fructose and glucose. The sugar chromatograms of peach juices showed similar profiles as given in Figure 1. The retention times of sucrose, glucose and fructose were typically as 8.760, 10.299 and 11.298 min, respectively. Direct injection of samples into HPLC was preferred where UV and RI detectors were connected in series. According to Castellari and co-workers (8), no significant difference was observed between directly injected samples and samples cleaned-up with strong anion exchange (SAX) cartridges prior to injection.

The sucrose content of aseptically packaged samples were significantly lower from that of Mersin (37.48±7.21 g/kg), Erdemli (35.23±5.46 g/kg) and Tepeköy (40.36±11.13 g/kg) samples ($p<0.05$). This might due to sucrose degradation at elevated temperatures during aseptic processing of peaches. The fructose content of the samples from Adana (23.03±5.39 g/kg) was significantly higher from that of Mersin (18.28±3.72 g/kg), Erdemli (18.37±2.54 g/kg) and Tepeköy (18.52±4.94 g/kg). There was no significant differences in glucose content of peaches among regions ($p<0.05$). According to AIJN standard for peaches, irrespective of culture variety, the ranges for glucose, fructose and sucrose are given as 7.5-25 g/kg, 10-32 g/kg and 12-60 g/kg, respectively. As given in Table 2, all samples from different regions fall in this range in terms glucose, fructose and sucrose.

Organic acid content of the juices of peach samples from different regions and aseptic packaging

The major organic acids found in peach are citric and malic acids. The HPLC chromatograms were similar for each analyzed peach samples with typical retention times as 9.261 min for citric acid and 10.935 min for malic acid (Figure 2). Our results were in good agreement with those of Castellari et al. (8) and Chinnici et al. (9).

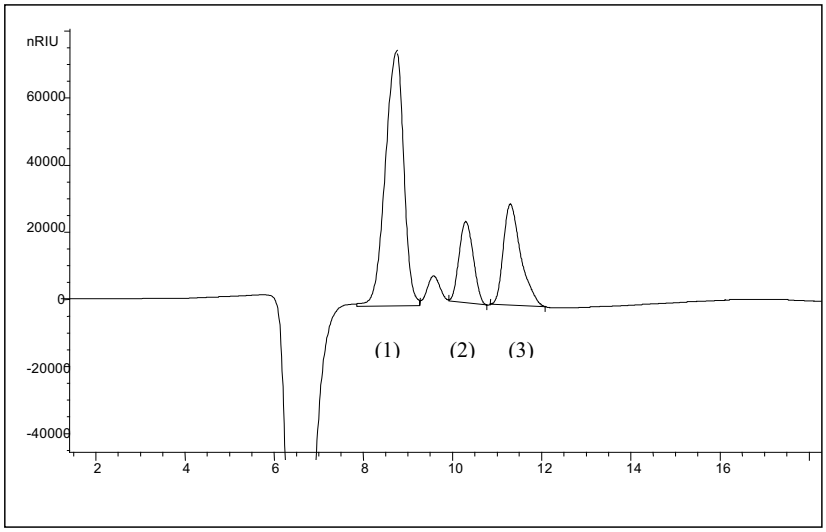

Figure 1. Carbohydrate chromatogram of peach sample collected from Adana at 23.08.04 (1) sucrose (RT 8.760 min), (2) glucose (RT 10.299 min), (3) fructose (11.298 min)

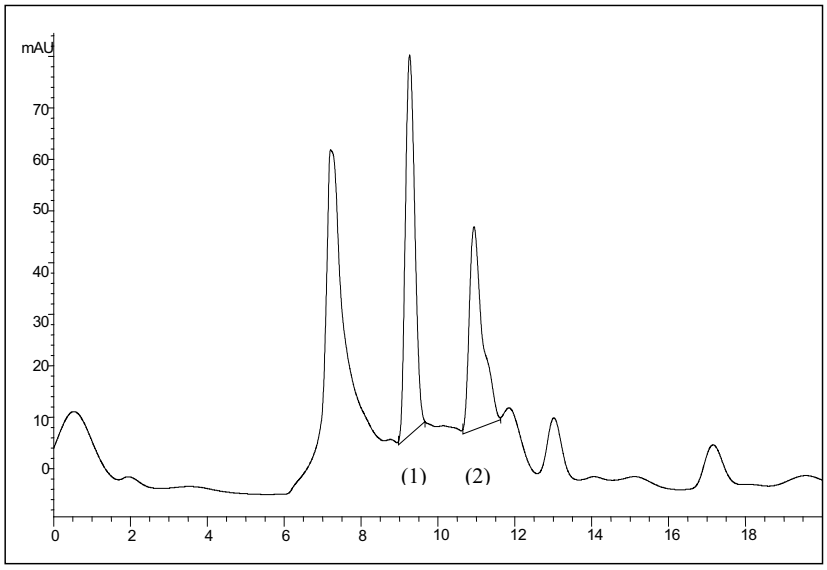

Figure 2. Organic acid chromatogram of peach sample collected from Adana at 23.08.04 (1) citric acid (RT 9.261 min), (2) malic acid (RT 10.935 min)

The malic acid content of aseptically processed peach juices was significantly higher from that of the samples from Mersin (3.09±0.69 g/kg) and Erdemli (3.01±0.96 g/kg) at $p<0.05$ level. The citric acid content of samples from Çandır (4.95±1.31 g/kg) was significantly higher than that of the peaches from Tepeköy (3.25±1.00 g/kg) and aseptically filled juices (3.21±0.60 g/kg) ($p<0.05$). Both malic and citric acid contents of the peaches from each region as well as aseptically processed peach juices were within the ranges given in AJIN (Table2).

Malic/citric acid ratio affects the taste. Malic acid has a much apparent acidic taste compared to citric acid. The higher this ratio the higher is the degree of acidity (27). The malic acid to citric acid ratio ranged from 0.74 to 0.85, on the average, for the peach samples from each region except that from Tepeköy with a higher ratio as 1.07. This ratio was much higher, 1.27 on the average, for the aseptically packaged samples. Versari et al. (3), reported that malic/citric acid ratios changed from 0.97 to 2.16 depending on variety. Colaric et al. (27),

reported somewhat higher malic/citric acid ratios ranging from 1.00-1.90. Byrne et al. (1), reported even higher malic/citric acid ratios as 2.02-2.79.

Total sugar/total organic acid ratio and level of citric acid have significant impacts on the perception of sweetness. Sugars to organic acids ratio is a common quality index (27). The total sugar/organic acid ratios were 9.45, 9.14, 9.27, 10.24, 8.07, 8.32 and 8,49 on the average for the samples from Adana, Mersin, Erdemli, Tepeköy, Çandır, Kızılbağ and aseptically packaged ones, respectively. This ratio was reported ranging from 5.38 to 10.74 by Colaric et al. (27). Versari et al. (3), reported this ratio changing between 12.16 and 13.42. Byrne et al. (1) evaluated this ratio as 11.09-14.18.

Amino acid content of the juices of peach samples from different regions and aseptic packaging

The major amino acid found in peach is asparagine. The other amino acids measured are aspartic acid, glutamic acid, serine, histidine, threonine, β-alanine, ɣ-aminobutyric acid (GABA), valine, methionine and ornithine. Their typical HPLC chromatograms for peach juice are given in Figure 3. Asparagine and serine co-eluted from the column.

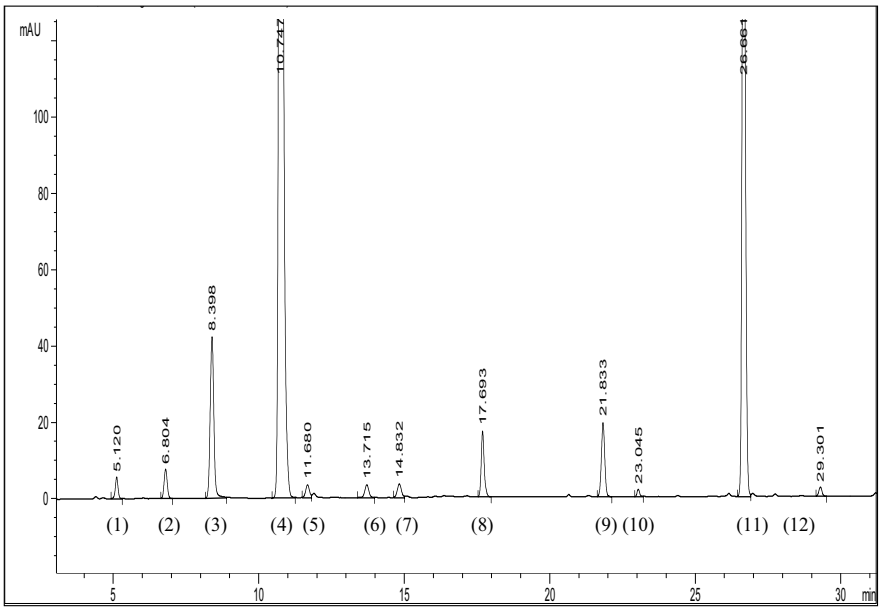

Figure 3. Amino acid chromatogram of peach sample collected from Mersin at 04.08.04 (1) Aspartic acid, (2) Glutamic acid, (3) Derivatization peak, (4) Asparagine+Serine, (5) Histidine, (6) Threonine, (7) β-Alanine, (8) γ-amino butyric acid (Gaba), (9) Valine, (10) Methionine, (11) Tryptophane (internal Standard), (12) Ornithine

The method of amino acid detection described by Hermosin et al.(13) was used with slight modifications. Peach juices were not isolated or purified prior to derivatization. Appropriate dilution factor at which no interference by juice matrix occurred was determined which corresponded to amount given in the materials and methods section. Furthermore, the derivatized samples were 15 fold diluted prior injection into HPLC. Also, some slight modifications were done for the first half of the gradient program in order to achieve better separation of the amino acid peaks by reducing the polarity of the mobile phase. The retention times for aspartic acid and glutamic acid were typically as 5.120 and 6.804 min, respectively. This result was contradictory to the chromatograms obtained by Hermosin et al. (13) where glutamic acid eluted prior to aspartic acid.

The asparagine-serine content was significantly higher in the peach samples from Adana 3170.35±872.80 mg/L) compared to that from Erdemli (2212.60±463.26 mg/L) ($p<0.05$). The asparagine-serine contents of the samples from each region and of aseptically processed juices were within the limits of given in AIJN (Table 2). The peaches from Mersin (9.47±5.44 mg/L) possessed significantly lower amounts of ornithine compared to aseptically processed samples (21.87±8.28 mg/L) ($p<0.05$). Although, the samples from each region and from aseptic process showed slightly higher ornithine contents, except that from Mersin, their mean values were within the range given in AIJN. Highest deviation from the upper limit was observed in processed juice samples (Table 2).

The samples from Çandır (14.70±2.38 mg/L) showed significantly lower threonine levels than that of samples from Kızılbağ (50.21±38.30 mg/L) ($p<0.05$). Threonine contents of all samples were within the ranges given in AIJN with some data slightly higher in samples from Kızılbağ and lower in that from Erdemli (Table 2).

γ -amino-butyric acid (GABA) content of peaches from Kızılbağ (98.93±31.22 mg/L) was significantly higher compared to that of samples from Adana (51.37±16.35 mg/L), Mersin (53.80±23.56 mg/L), Erdemli (46.80±12.99 mg/L) and Tepeköy (56.31±17.23 mg/L) ($p<0.05$). GABA values were within the limits of AIJN (Table 2).

Aseptically processed peach juices (106.32±17.15 mg/L) possessed significantly higher amounts of aspartic acid compared to the regions except Kızılbağ (75.85±16.35 mg/L) ($p<0.05$). This might be due to degradation of some asparagine to aspartic acid by elevated temperature during processing. Although the mean values of aspartic acid content was within the limits given by AIJN, the peach samples from all regions investigated showed aspartic acid levels below the limits except that from Kızılbağ (Table 2).

Adana (68.95±32.38 mg/L) region possessed peaches with significantly lower valine content compared to that from Mersin (131.59±44.53 mg/L) and Erdemli (160.19±63.24 mg/L), as well aseptically processed samples (148.54±44.65 mg/L) ($p<0.05$). The amount of valine in peach samples was well above the limits given in AIJN (5-50 mg/L) for all regions. Only samples from Adana with lowest levels of valine measured were slightly below the upper limit.

No significant differences among regions were found for methionine, alanine, glutamic acid, histidine contents in peaches. According to AIJN, alanine content of peach should be between 40-300 mg/L. The amount of alanine was below 40 mg/L in all peach samples regardless of the region they belong to. Only peaches from Kızılbağ possessed slightly higher amounts of this amino acid (Table 2).

CONCLUSION

Among the individual sugars measured only the fructose content showed some differences among the regions. While malic acid content did not differ among regions, citric acid content differed only for two regions. The malic/citric acid ratio, therefore, might be a better indicator in terms of organic acids measured in peach samples from different regions which was almost the same for each region except Tepeköy with somewhat a higher value. Also, the total sugar/total organic acid ratio is a good indicator which was around 10 for the samples from Adana, Mersin, Erdemli, Tepeköy and around 8 for Çandır and Kızılbağ.

Methionine, alanine, glutamic acid and histidine contents did not differ statistically in samples from different regions. The most striking results were obtained for valine and alanine contents. Valine values measured were higher whereas alanine values, on the contrary, were lower than the acceptable ranges given in the AIJN standard.

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