

INVESTIGATION OF 5-HYDROXYMETHYL-2-FURALDEHYDE AND 2-FURALDEHYDE COMPOUNDS IN FRUIT JUICES

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ABSTRACT

Maillard reactions are responsible for reducing the nutritional value of foods. Hydroxymethylfurfural which is the intermediate products of Maillard reaction is the most important quality criteria in fruit juices. Our aim was to determine the levels of 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (F) compounds in 100 commercial fruit juice samples (apple juice, apricot nectar, cherry juice and peach nectar) of five different brands (A, B, C, D, and E) sold in Ankara, Turkey. HMF and F compounds were determined by high-performance liquid chromatography (HPLC) with diode array detector (DAD). The HMF and F assays were linear in broad concentration ranges (HMF: $R^2 > 0.999$, F: $R^2 > 0.994$). Recovery values of HMF and F were calculated as 101.8% and 99.1%, respectively. Limit of detection (LOD) and limit of quantification (LOQ) values of HMF were determined as 0.0017 mg/L and 0.0055 mg/L, respectively. These values were determined as 0.0018 mg/L and 0.0059 mg/L for F. HMF and F were determined in all of the samples. Also, HMF levels of all samples were higher than F levels of samples. The minimum and maximum HMF and F levels were determined as 63.89 – 162.27 mg/L and 0.19 – 4.85 mg/L, respectively. Our data revealed that HMF levels in fruit juice samples were higher than maximum allowed value set by Turkish Standard Institute (TSI) (10 mg/L). No value has been established in the TSI for F compound in fruit juices or nectars.

Keywords: Fruit juice, 5-hydroxymethyl-2-furaldehyde, 2-furaldehyde, high-performance liquid chromatography.

MEYVE SULARINDA 5-HİDROKSİMETİL-2-FURALDEHİT VE 2-FURALDEHİT BİLEŞİKLERİNİN ARAŞTIRILMASI

ÖZET

Maillard reaksiyonları gıdaların besinsel değerini düşürebilir. Maillard reaksiyonu ara ürünlerinden olan hidroksimetilfurfural meyve sularında en önemli kalite kriteridir. Çalışmada Ankara'da tüketime sunulan beş farklı markanın (A, B, C, D, E) 100 adet ticari meyve suyu örneğinde (kayısı, elma, vişne, şeftali) 5-hidroksimetil-2-furaldehit (HMF) ve 2-furaldehit (F) bileşiklerinin düzeylerinin belirlenmesi amaçlanmıştır. HMF ve F bileşiklerinin analizleri diyet dizinli dedektörlü, yüksek performanslı sıvı kromatografisi (HPLC-DAD) kullanılarak gerçekleştirilmiştir. HMF ve F deneyleri geniş konsantrasyon aralıklarında doğrusal sonuç vermiştir (HMF: $R^2 > 0,999$, F: $R^2 > 0,994$). HMF ve F'nin ortalama geri kazanımları sırasıyla %101,8 ve %99,1 olarak bulunmuştur. HMF'nin teşhis sınırı (TS) ve tayin alt sınırı (TAS) değerleri sırasıyla 0,0017 mg/L ve 0,0055 mg/L olarak tespit edilmiştir. Bu değerler F için sırasıyla 0,0018 mg/L ve 0,0059 mg/L olarak belirlenmiştir. Minimum ve maksimum HMF ve F seviyeleri sırasıyla 63,89-162,27 mg/L ve 0,19-4,85 mg/L olarak tespit edilmiştir. Elde edilen sonuçlara göre meyve sularındaki HMF düzeyleri Türk Standartları Enstitüsünde (TSE) belirtilen maksimum izin verilen düzeyin (10 mg/L) üzerinde bulunmuştur. Meyve sularında veya nektarlarda TSE'de F bileşiği için bir değer belirtilmemiştir.

Anahtar kelimeler: Meyve suyu, 5-hidroksimetil-2-furaldehit, 2-furaldehit, yüksek performanslı sıvı kromatografisi.

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INTRODUCTION

Fruit juices have an important role in human nutrition and they are sources of many nutrients and energy (1). Processed fruit juices may contain 5-hydroxymethyl-2-furaldehyde (HMF) and 2-furaldehyde (F) compounds that are known as an indicator of product quality. HMF and F are related to color and flavor changes in processed fruit juices (2). Generally, these compounds are not found or are found in a few in fresh unheated juices (3, 4). HMF and F are formed during thermal processing of production (heating and pasteurization) steps and storage (2). A thermal process is an important processing step in food production due to the destruction of microorganisms and improves sensory properties such as color, taste, and aroma (5). HMF is formed during the Maillard reaction or caramelisation which are related to the thermal process applied to foods containing, particularly carbohydrates (5, 6). HMF and F are found in many carbohydrate-containing foods such as fruit, coffee, milk and cereal-based baby foods, honey, fruit juices, syrups, tomato puree, ketchup and jam (7-9).

HMF has various side-effects on health. Hazards from exposure to high-level HMF were cytotoxic and cause irritation to eyes, upper respiratory tract, skin and mucous membranes (5). Whether consumption of foodborne HMF poses a potential health risk for humans or not is arguable (10). HMF is present at high levels in several foods and can be metabolized to 5-sulfoxymethylfurfural, which is mutagenic and carcinogenic. This reactive metabolite could be responsible for renal tubule damage (11). On the other hand, Abraham et al. (8) assessed that there are limited studies related to the HMF toxicity, and stated that a critical effect is not

obviously specified. Although there are contradictory studies on the possible carcinogenicity of HMF, risk assessment of HMF should be improved by tissue-specific DNA studies and in vivo studies on genotoxicity of HMF. Dietary exposure studies of HMF are needed to assess dietary intake for a different population. At the present time, no values have been established in the Turkish Food Codex (TFC) for HMF and F compounds levels in fruit juices or nectars (12).

In the Turkish Standard Institute (TSI), the levels of HMF are regulated as 10 mg/L in apple juice, peach nectar, apricot and cherry nectar (13-16). Our aim was to investigate the presence of HMF and F compounds in apple juice, peach nectar, apricot and cherry nectar samples of five different brands sold in Ankara markets, in Turkey.

MATERIALS AND METHODS

Samples

In this study, one hundred fruit juice samples (apricot nectar, apple juice, cherry nectar and peach nectar) were collected and analyzed from different brands (A, B, C, D, and E) in Ankara, Turkey in 2014. Samples were kept at +4 °C. For sampling procedure, having a different serial number and the production date is important in terms of realizing the persistence of quality at the production process. The package of samples was opened just before the analysis.

Reagents and standards

5-hydroxymethyl-2-furaldehyde and 2-furaldehyde (Sigma-Aldrich, St. Louis, MO, USA) were used as analytical standards. The oxalic acid (C₂H₂O₄) was obtained from Sigma-Aldrich (Steinheim, Germany). Methanol (CH₃OH) was purchased from Merck Chemical (Darmstadt, Germany). Potassium ferrocyanide (Carlo Erba Chemical, Milano, Italy) and zinc acetate (Panreac,

Barcelona, Spain) were used in the preparation of Carrez solutions. Deionized water was used throughout the experiments (Millipore Simplicity 185, Molsheim, France). All of the reagents were of analytical grade or HPLC grade. Stock solutions of HMF (100 µg/mL) and F (10 µg/mL) were prepared in deionized water. The calibration curve was obtained using a series of dilutions containing different levels for HMF (0.05-75 µg/mL) and F (0.02-4 µg/mL) stock solution.

Sample preparation for potential HMF and F extraction

The extraction and determination procedures for the analysis of potential HMF and F are based on the method described by Guerra-Hernandez et al. (17). Briefly, 10 mL of fruit juice sample and 5 mL of 0.3 M oxalic acid were transferred to centrifuge tubes and vortexed well (Firlabo, Lyon, France). The mixture was heated in a water bath for 25 min. (Memmert WB 10, Schwabach, Germany). After cooling, 2 mL of each Carrez I (potassium ferrocyanide, 150 g/L) and Carrez II (zinc acetate, 300 g/L) added and vortexed well. Then, the mixture was stirred on an orbital shaker for 10 min. (Biosan, MR-1, EU) and centrifuged at 500 x g for 5 min (MSE, Mistral 1000, UK). After centrifuging and filtering with 0.20 µm filter (Sartorius, Goettingen, Germany), the supernatants were injected into the HPLC system. Injection volumes of sample and standard were 20 µL.

HPLC Conditions for potential HMF and F analysis

The potential HMF and F were analyzed by the HPLC (Agilent Series 1200, Santa Clara, CA, USA) using a diode array detector (Agilent G1314B VWD Series). Detection of potential HMF and F were performed at 284 nm. HPLC separation was carried out using the mobile phase of methanol/water (17.5:82.5, v/v) at a flow rate of 1 mL/min. Spherisorb (Waters, Dublin, Ireland) ODS2 (250 mm×4.6 mm i.d., 5 µm) column as the stationary phase was used in separation. The mean retention time for HMF and F standards were 7.6 min and 12.4 min, respectively.

Statistical analysis

One-way ANOVA and One-sample t-tests were conducted for the statistical comparison (18).

RESULTS

The mean recoveries of HMF and F were found as 101.8% and 99.1%, respectively. The precision of the method was assessed by Intra-day and inter-day repeatability of responses after replicate injection (n=5) of standard solutions (0.05 µg/mL). The values of percent relative standard deviation (RSD %) of Intra-day and inter-day precision of HMF and F were calculated as 1.45% - 2.68%, and 0.35% - 3.9%, respectively (Table 1). The linear regression equations of HMF and F were determined as $y = 127.26x + 54.483$ and $y = 84.587x + 10.263$, respectively.

Table 1. Method performance of HMF and F

Analyte	Matrix	LOD mg/L	LOQ mg/L	Recovery Range %	RSD, % (n=5)
HMF	Fruit juice	0.0017	0.0055	101.8	3.90
F	Fruit juice	0.0018	0.0059	99.1	0.35

A total of 100 samples of fruit juice were analyzed and HMF and F were present in all of the examined samples. The levels of HMF and F in fruit juice samples were shown in Table 2 and Table 3, respectively. All of the analyses were performed in three times for each sample. The results of the HMF and F analyses were evaluated in accordance with the maximum limit value (10 mg/L) for apple juice, peach nectar, apricot and cherry nectar established by TSI. The minimum and maximum HMF levels of

samples were determined as 63.89-162.27 mg/L. In addition, potential F concentrations of samples were ranged from 0.19 to 4.85 mg/L. The mean HMF values (\pm SE) of A, B, C, D and E brands were determined to be 85.82 \pm 3.51, 97.60 \pm 3.85, 101.08 \pm 4.50, 105.84 \pm 4.35 and 104.27 \pm 4.51 mg/L, respectively. Mean F values (\pm SE) of A, B, C, D and E brands were also determined to be 1.11 \pm 0.16, 1.34 \pm 0.12, 1.24 \pm 0.09, 1.15 \pm 0.11 and 1.46 \pm 0.24 mg/L, respectively.

Table 2. HMF values (mg/L) of fruit juice samples

Brands	N	Mean \pm SE (mg/L)	Min (mg/L)	Max (mg/L)
Apricot	5	83.73 \pm 5.40	73.69	103.39
Apple	5	94.97 \pm 7.79	70.54	111.92
A Cherry	5	80.56 \pm 4.58	70.46	94.02
Peach	5	84.02 \pm 9.70	63.89	119.36
Total	20	85.82 \pm 3.51 ^a	63.89	119.36
Apricot	5	92.91 \pm 6.49	74.81	109.44
Apple	5	106.36 \pm 10.74	71.41	133.42
B Cherry	5	95.72 \pm 8.37	74.03	120.82
Peach	5	95.39 \pm 5.20	77.32	107.39
Total	20	97.60 \pm 3.85 ^b	71.41	133.42
Apricot	5	89.70 \pm 7.19	65.63	107.83
Apple	5	111.37 \pm 12.56	70.33	140.17
C Cherry	5	103.89 \pm 5.28	92.04	120.51

	Peach	5	99.33±9.24	80.43	132.66
	Total	20	101.08±4.50 ^b	65.63	140.17
	Apricot	5	109.66±12.43	65.28	134.02
	Apple	5	99.97±11.49	78.70	141.07
D	Cherry	5	108.58±5.71	93.24	127.49
	Peach	5	105.15±5.14	86.14	115.79
	Total	20	105.84±4.35 ^b	65.28	141.07
	Apricot	5	103.18±17.02	75.37	162.27
	Apple	5	100.16±5.38	85.69	113.96
E	Cherry	5	108.58±5.71	93.24	127.49
	Peach	5	105.15±5.14	86.14	115.79
	Total	20	104.27±4.51 ^b	75.37	162.27

^{a-b}: within a column, means with different letters are significantly different from each other at $p<0.01$.
The difference between HMF values of juices types for each brand was not significant ($p>0.05$).

Our data revealed that HMF levels of all samples were determined as higher than F levels. For HMF, the difference between brands was statistically significant in fruit juice samples ($p<0.01$) while the difference between HMF values of juices types for each brand was not significant ($p>0.05$). The difference between F values of brands

was not significant ($p>0.05$). HMF values of cherry juice ($p<0.05$) and F values of cherry juice ($p<0.001$) and apricot nectar ($p<0.05$) between brands were statistically different. Mean HMF value of A brand is lower than the other groups. HMF values of brands were higher than the TSI limit value (10 mg/L).

Table 3. F values (mg/L) of fruit juice samples

Brands	N	Mean±SE (mg/L)	Min (mg/L)	Max (mg/L)
Apricot	5	1.01±0.17	0.48	1.40
Apple	5	1.98±0.34	1.16	3.15
A Cherry	5	0.40±0.05	0.21	0.51
Peach	5	1.04±0.18	0.64	1.66
Total	20	1.11±0.16	0.21	3.15
Apricot	5	1.54±0.18	1.11	2.09
Apple	5	1.76±0.17	1.35	2.35
B Cherry	5	0.62±0.11	0.19	0.76
Peach	5	1.42±0.18	0.81	1.89
Total	20	1.34±0.12	0.19	2.35
Apricot	5	0.99±0.08	0.75	1.19
Apple	5	1.64±0.18	1.04	1.98

C	Cherry	5	1.25±0.16	0.72	1.68
	Peach	5	1.09±0.14	0.66	1.43
	Total	20	1.24±0.09	0.66	1.98
D	Apricot	5	1.51±0.24	0.92	2.31
	Apple	5	1.12±0.14	0.71	1.49
	Total	20	1.15±0.11	0.30	2.31
E	Apricot	5	2.59±0.72	0.93	4.85
	Apple	5	1.31±0.23	0.68	1.75
	Total	20	1.46±0.24	0.30	4.85

DISCUSSION

HMF levels in different food samples were determined by several studies in Turkey. But, the quantifying studies about HMF in commercial fruit juices are limited. Altunöz Erdoğan et al. (19) analyzed different fruit juices (orange nectar, grape juice, apricot and cherry nectar) and found that lower HMF levels in orange nectar and grape juice, while these researchers found higher HMF levels in apricot and cherry nectar compared to TSI value. Tüfekçi and Fenercioğlu (20) estimated that the HMF levels of some commercial fruit juices (apple, pomegranate, orange and grape juice) were ranged from 0.4 to 27.4 mg/L and they stated that HMF levels of fruit juices were below according to the maximum levels established by TSI, except two samples in the pomegranate and grape juices as 27.4 and 24.4 mg/L, respectively. Effect of high-temperature heat process or inappropriate storage temperature on the formation of high

HMF levels was expressed by Tüfekçi and Fenercioğlu (20).

In the current study, the HMF levels were found to be in the range of 63.89 to 162.27 mg/L in the tested commercial fruit juice samples. These HMF levels in commercial fruit juice samples were higher compared with the Tüfekçi and Fenercioğlu (20).

Akkaya and Karataş (21) found that HMF values of apple juices as 1.77-7.73 mg/L. Kuş et al. (22) determined HMF concentrations of seven fruit concentrates and boiled juices in all samples as in the range of 0.4-4.5 ppm and 12.8-3500 ppm, respectively. The formation of HMF in fruit juices is affected several processes such as concentrations of fruit juices, dehydration of fruits or storage at a higher temperature (22). Oral et al. (23) determined HMF contents of fruit juices concentrates, honey and molasses (pekmez) and they noted that HMF

contents were higher than TSI limits (10 mg/L).

In several countries, several studies were previously reported concerning HMF contents in fruit juices. Santini et al. (24) established that HMF levels ranging from 0.24 to 28.61 mg/L in apple-based nectars and 0.06 to 18.12 mg/L in apple juice, and this levels could be attributed to strong thermal treatment on the fresh apple during processing steps. Vorlová et al. (7) reported mean levels of HMF as 0-2.8 mg/kg in a total of 12 orange juice samples examined in the Czech Republic. Matić et al. (25) indicated that mean HMF level as 9.89 ± 12.1 mg/kg in 20 apple juice in Serbia and HMF levels of three apple juice sample were higher than the maximum allowed HMF levels (20 mg/kg) established by Serbian legislation. Jafarnia et al. (26) analyzed 40 traditionally and 12 industrially date syrup and they found that HMF values of fresh traditional and industrial date syrups ranged from 1000 to 2675 mg/kg and 12 to 456 mg/kg, respectively. Lee et al. (27) used HPLC method for the determination of 5-hydroxymethylfurfural in fruit juices in Malaysia and they found 5-HMF in all samples ranging from 0.08 to 91.5 mg/L. They noted that the HMF values of tropical juices were higher. Jalili and Ansari (28) mentioned that HMF contents ranged from 11.42 mg/kg to 39.24 mg/kg in 8 fruit juices samples. Teixido et al. (29) analyzed HMF content of apple and orange juices and they noted that the maximum HMF content of apple and orange juices were

3.5 mg/kg and 10.6 mg/kg, respectively. Zhang et al. (30) determined HMF contents in foods consumed in China. They found that HMF values ranged from n.d. to 8.6 mg/kg, and found mean values as 1.7 mg/kg in fruit juices.

These HMF levels in fruit juice samples determined by several researchers were lower when compared with the value reported in this study. Research results may be varying because of technological differences, used materials, regional difference and different storage conditions. Heat treatment is one of the most important factors affecting the quality of fruit juices. In food processing, furfural compounds are occurred due to the high-temperature applications. In addition, the formation of furfural is affected by unsuitable storage temperature of fruit juices.

Generally, quantifying studies about furfural compounds in fruit juices are limited. HMF and F are a very important quality indicator in beverage processing. There is not enough information related to negative direct effects of HMF on health. Some studies are reported that metabolic product of HMF had adverse health effects.

Finally, the findings of the present study indicate that the monitoring of HMF in fruit juices is important due to the quality of products.

CONCLUSIONS

An important problem caused by the heating process is the occurrence of some compounds that do not naturally exist in the foods. Furfural compounds occur during the non-enzymatic browning reactions and they are most known Maillard reaction products that used as an indicator to examine the effects of heat processing on food. The presence of HMF and F, called furfural compounds, is accepted as a freshness and quality parameter in the foodstuffs. For this purpose, these compounds are analytically controlled in order to evaluate the quality of food processing and organoleptic properties of the final products. The application of the cooling process after the temperature cycle in the production will also be beneficial due to the ensure quality of the final product. Non-enzymatic browning reactions could not just cause quality loss such as product appearance but also affect food safety due to the formation of HMF. It was demonstrated that high concentration of HMF has possible negative effects. From production to consumption precautions must be taken and usual controls must be carried out for food safety and consumer health. Production technologies and storage conditions could be suggested to improve in commercial fruit juices.

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