DETERMINATION OF 5-HYDROXYMETHYLFURFURAL IN APPLE JUICE

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Abstract Hydroxymethylfurfural (5-HMF) was determined by a HPLC method in 20 samples of apple juice. The samples were extracted and the extracts were then cleaned up on silica gel solid-phase column. Then 5-HMF was determined by reversed-phase liquid chromatography using a C_{18} column and a photodiode array (DAD) detector, using the mixture water/acetonitrile/60% perchloric acid (990:10:1, v/v/v) as the mobile phase with a flow rate of 1.0 ml/min. These chromatography conditions have shown good separation of 5-HMF and patulin in noticeably shorter time than previous results. The presence of 5-HMF was determined in all of the examined samples. In 17 (85%) samples concentration of 5-HMF was less than 20 mg/kg, while in 3 (15%) samples concentration was higher than maximum prescribed value by Serbian legislation (20 mg/kg).

Key words: *5-HMF, HPLC, apple juice*

INTRODUCTION

5-Hydroxymethylfurfural (5-HMF) is an aldehyde, which can be used as an indicator of apple juice quality, since the presence of 5-HMF is considered as an indication of quality deterioration. 5-HMF is characteristic flavor compound of the Maillard reaction, and it is the result of complex series of reactions between amino acids and reducing sugars (hexoses). 5-HMF is practically not present in fresh food, but it is naturally created in sugar containing food during heat treatments. Formation of 5-HMF in foods varies with processing and storage conditions and is especially dependent on temperature and pH. Suitable conditions for the formation of 5-HMF are: high concentration of saccharides (mainly hexoses), lower pH value, presence of organic acids and low water activity. Concentration of 5-HMF also increases during the heating or storage processes (Lansalot-Matras, 2003), and it is commonly found in honey, fruit juices, UHT milk, coffee and dried fruit. It is not clear whether a human exposure to 5-HMF represents a potential health risk. Ulbricht et al. (1984) have shown that, at high concentrations, 5-HMF is cytotoxic, causing irritation to eyes, upper respiratory tract, skin and mucous membranes. They have also determined an oral LD_{50} of 3.1 g/kg of body mass in rats. Data from epidemiological

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studies or case reports on potential association of 5-HMF with cancer risk in humans is not available.

Control of 5-HMF in apple juice in Serbia is obligatory from 2010, since the older legislation ("Službeni list SRJ", br. 33/95) did not prescribe control. In accordance with the official legislation ("Službeni glasnik RS", 27/10) 20 mg/kg is allowed limits for 5-HMF in apple juice. The International Federation of Fruit Juice Processors (IFFJP) has recommended maximum concentrations of 5-10 mg/l and 25 mg/kg in fruit juices and concentrates, respectively (Wagner, 2006); and the European Union has set a limit of 20 mg/kg 5-HMF for juices made for children (FPA, 2006).

Numerous analytical methods have been developed for the determination of 5-HMF in various food products. Spectrophotometric methods have been used for many years and are often the official method for the determination of 5-HMF in food. In general, these methods lack specificity because the total amount of furanic aldehydes is determined (Theobald, 1998). The chromatographic techniques in use are based on liquid chromatography (Fuleki, 1993), gas chromatography (Hernandez et al., 1988), ion exclusion chromatography (Richardson, 1992), and micellar electrokinetic chromatography (Corradini, 1992).

One of the most used method for the quantitative determination of 5-HMF is liquid chromatography with UV detection.

5-HMF and patulin exibit similar chromatographic properties owing to their chemical structures (Figure 1), and therefore, many authors have investigated the possibility of simultaneous determination of these contaminants in apple juice (Gokmen, 1999).

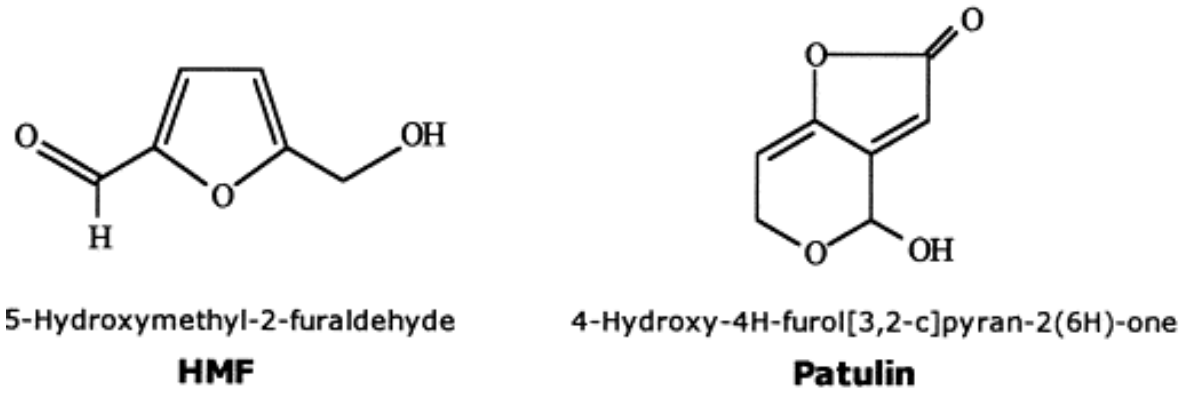

Figure 1. Chemical structures of 5-HMF and patulin

Patulin is a mycotoxin mainly found in apples and apple products, and has become one of the most important quality criteria for apple juice. In the analysis of patulin in apple tissues and derived products, the most common interfering agent is 5-HMF, which affects the quantification of patulin. This interfering agent must be considered in the analytical methodologies in general, since 5-HMF levels are two to three times higher than the levels of patulin normally detected (da Silva, 2007).

The aim of this work is to investigate the content of 5-HMF in various apple juice samples, in order to give an overview of the actual concentration of this compound in apple juice.

1. MATERIALS AND METHODS

1.1. Materials

Samples of apple juice were collected from supermarket in Novi Sad, Serbia. A total of 20 samples were produced in Serbia. Among the 20 analyzed samples, 10 were 100% fruit juice, and other 10 samples were approximately 50% fruit juice. All of these samples were in Tetra Pack containers of 1000 ml. Samples were stored at room temperature and analyzed before expiration dates.

1.2. Reagents

Acetonitrile, ethyl acetate and n-Hexane (HPLC grade), ethanol, acetic acid (extra pure grade) anhydrous sodium sulfate, anhy-

drous sodium hydrogen carbonate, perchloric acid 60% and sand (p.a.) were purchased from Merck (Darmstadt, Germany). The HMF and patulin standards were obtained from Sigma-Aldrich. Water was ultra pure (Milli-Q from Millipore, USA)

Stock solution of 5-HMF was prepared by dissolving 5 mg in 25 ml of ethyl acetate, then diluting this solution 1:50 (v/v) by ethyl acetate to obtain a final 5-HMF concentration of 0.2 mg/ ml. A 100 µl of these stock solutions was transferred into a 10 ml volumetric flask and evaporated to dryness under a stream of nitrogen at room temperature. The residues were immediately dissolved in 10 ml of water (pH 4.0) acidified with acetic acid. Working standards were prepared by appropriate dilution of these solutions with acidified water (pH 4.0). Stock solution of patulin was also prepared at a concentration of 0.2 mg/ml. The standard mixture of 5-HMF and patulin was prepared from stock solutions.

1.3. HPLC-DAD

The HPLC instrument was an Agilent 1200 system equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, a vacuum degasser, an auto sampler and Agilent column (Eclipse XDB-C18, 1.8 µm, 4.6 x 50 mm). The mobile phase consisted of an isocratic mixture of water/acetonitrile/60% perchloric acid (990:10:1, v/v/v) for 3 min, with a flow rate of 1.0 ml/min. Ten microliters of standards and samples was injected onto the HPLC column. The spectra was recorded at 276 nm. Identification of 5-HMF was done by comparing the retention times and spectra of 5-HMF from samples and standards.

1.4. Analysis of 5 – HMF in apple juice

5- HMF extraction and the cleanup procedure were performed according to the method described by Arranz (Arranz et al, 2005). Around 10 ml of apple juice was extracted in extraction tube with ethyl acetate extraction solvent (ethyl acetate /n-hexane, 60:40, v/v), sodium sulfate, sodium hydrogen carbonate and sand. After extraction, extract was cleaned up on silica gel solid-phase column (SPE, Supelco, Bellefonte, PA). The purified extract was evaporated to dryness under stream of nitrogen, redissolved in aqueous acetic acid solution (pH 4). The redissolved samples were filtered through a membrane filter (0.45 µm) and the filtrate was transferred to an HPLC vial.

2. RESULTS AND DISCUSSION

The standard mixture of 5-HMF and patulin was used in order to establish optimum conditions for separation on Eclipse XDB-C_{18} column. The Figure 2. illustrates the se-paration of 5-HMF and patulin using water/acetonitrile/60%perchloric acid (990:10:1, v/v/v) as the mobile phase at a flow rate of 1.0 ml/min. These chromatography conditions have shown good separation of 5-HMF and patulin in noticeably shorter time than previous results. Jalali et al. (2010) demonstrated that when using C_{18} (250 x 4.6 mm, 5 µm) column with acetonitrile-water (1:10, v/v) as mobile phase, 5-HMF and patulin were eluated for 8.01 min and 9.78 min, respectively. Gokmen at al. (1999) have shown faster elution and separation of 5-HMF (6.1 min) and patulin (8.7 min), with the same mobile phase, on shorter C_{18} column (150 x 4 mm, 5 µm). Iha et al. (2009) used C_{18} column (Shimpack, 200x 4.6 mm, 5 µm) with water and ethanol in gradient elution, and achieved separation of 5-HMF at 15.8 min and patulin at 17.4 min.

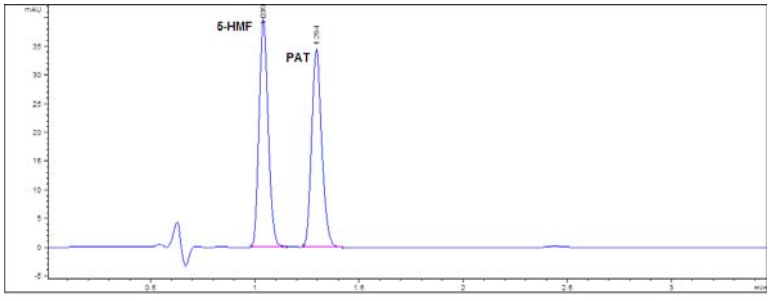

Figure 2. HPLC chromatogram of standard mixture of 5-HMF and patulin

Results of the HPLC analysis of apple juice for 5-HMF are presented in Table 1.

Table 1.
Result of analyzed apple juice for the presence of 5-HMF

Sample type	No.	5 – HMF <20 mg/kg	5 – HMF >20 mg/kg	Maximum (mg/kg)	Mean ± SD
100 % apple juice	10	9 (90%)	1 (10%)	47.8	9.46 ± 14.8
50 % apple juice	10	8 (80%)	2 (20%)	38.5	11.5 ± 11.0
Total	20	17 (85%)	3 (15%)	47.8	9.89 ± 12.1

From these results it can be noted that chromatography conditions described in this paper provide very good separation and fast analysis.

A total of 20 samples of apple juice were analyzed and 5-HMF was present in all of examined samples. Among 10 examined samples from the category of 100% apple juice, 5-HMF was found in 9 samples at concentration less than 20 mg/kg. Only one sample from this category had HMF content above the 20 mg/kg, which is the maximum prescribed value in Serbian legislation ("Službeni glasnik RS", 27/10). The maximum level found in 100% apple juice was 47.8 mg/kg, while the mean content of 5-HMF was 9.46 ± 14.8 mg/kg.

In the category of 50% apple juice, 8 samples 5-HMF have shown concentration bellow than 20 mg/kg. In 2 samples HMF was detected in the amount higher than 20 mg/kg, and these samples are not in accordance with the Serbian legislation. The mean level of 11.5 ± 11.0 mg/kg of 5-HMF found in 50% apple juice was slightly higher than in samples with 100% apple juice.

Finally, of the 20 examined samples of apple juice, in 3 (15%) samples content of 5-HMF was higher than maximum allowed value set by domestic legislation.

Until this year maximum levels of 5-HMF in apple juices have not been prescribed by Serbian legislation. Results of this study indicate the importance of future monitoring of 5-HMF in apple juices.

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