

DETERMINATION OF HYDROXYMETHYLFURFURAL CONTENTS OF SOME APPLE JUICES ON THE MARKET BY HPLC METHOD

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Abstract

Hydroxymethylfurfural (HMF) were formed when foods that contains sugar stored at improper temperatures or heat treatment applied to high temperature during production. In this research, the conformities of some of the apple juices sold in the Turkish market were estimated according to Food Regulations . For this purpos eight different companies essays in markets of Istanbul were coolected randomly in order to analysis of PH, hydroxylmethylfurfural (HMF) and water soluble dry matter of apple juices . HMF was analysed in the each sample was carried out by using High Performance Liquid Chromatography (HPLC). Result indicated that minimum and maximum values for HMF. PH, water soluble dry matter of apple juices were estimated as 1.77-7.73 mg/L, 2.88-3.83 and 11.25-11.90% respectively .

Keywords: *Apple juice, Hydroxymethylfurfural (HMF), HPLC, PH*

INTRODUCTION

There is an increased concern in the apple juice processing industry about quality maintenance and the avoidance of product

adulteration . One of the major issues of adulteration is the use and substitution of fresh juice with apple juice concentrate.

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Since the production of concentrated apple juice involves heating, evaporation and storage, any changes in the compositional profile of phenolic compounds could be potentially used as a marker for monitoring any adulteration and, hence, to provide a reliable tool to distinguish between fresh and concentrated apple juices [1].

5-hydroxymethylfurfural (HMF) is an important quality criteria in fruit juices. The presence of HMF is considered as an indication of quality deterioration. It is formed as a result of dehydration of ketopentoses, particularly in acidic or high-temperature environments [2].

HMF consists of an aromatic alcohol, an aromatic aldehyde and the furan ring. Molecular weight of HMF 126.11 g / mol, density 1.29 g / cm³, in the form of the chemical formula C₆O₆ H₃. HMF is an intermediate product of the well-known Maillard Reaction or is formed as a result of dehydration of hexoses under acidic environments. HMF is used as an index for the formation of juices, milk, honey, cereals, determination of storage time in many products such as jams, in order to

understand whether or chemical appropriate heat treatment [3].

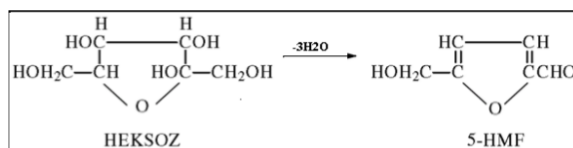


Fig. 1. Heating results in the acidic environment of 5-HMF formation of hexoses [3].

HMF is not present in fresh fruits, but it is naturally generated in sugar-containing food during heat-treatments such as drying or cooking. To date, the toxicological relevance of 5-HMF is not clearly documented. Nevertheless, 5-HMF in foodstuffs has attracted much interest because it exhibits mutagenic and DNA strand-breaking activity. Besides that, cytotoxic, nephrotoxic, carcinogenic, genotoxic are among other *in vitro* activities attributed to 5-HMF [4].

Maillard reaction (MR) may occur during food processing and/or storage, particularly at high temperatures, in carbohydrate-containing and lysine-rich protein foods. The reductor sugars and lysine are the main compounds involved in the initial states of the MR. In advanced states of MR,

undesirable compounds such as furfurals can be found [5].

If there is a level above a certain amount of HMF, the color is browning, significant deterioration in terms of taste and odor, lead to a reduction in nutrient levels of the product it is caused. For this reason, the possibility of marketing the product can eliminate partially or completely. Therefore, HMF in foods that are allowed are limited [6]. Apple juices are indicated that maximum limit of 10 mg /L [7].

HMF can be made of different analysis methods for quantitative analysis developed in the juice. These methods can be listed as spectrophotometric method, high pressure liquid chromatography (HPLC) method and the micellar capillary electro cromotografi (MECC) method. HPLC method is widely used in HMF analysis. The reason is that it is performed more quickly and easily than other methods. HPLC methods are routine and inexpensive way. It does not require any preparation except for sample dilution and filtration [8]. Purposes of this research were to investigate amount of HMF in apple juice and in Turkish markets in order to prevent

maintenance and the avoidance of apple juice adulteration.

MATERIALS and METHODS

Apple juice samples were collected and selected radomly from the dominant firm productin Turkish market. The Company 1/1, 1/4 and 1/5 L-volume products were selected, each firm was represented one of the three different sample volumes.

Apple juice of different companies that make up packaging material research specimens of information is given in Table 1.

Product code	Production and Expiration date	Amount of product
A	17.09.14/17.09.15	200 ml
B	-/21.08.2016	1 L
C	23.12.14/23.12.15	200 ml
D	13.10.14/13.10.15	200 ml
E	20.06.15/20.06.16	200 ml
F	-/27.05.2017	250 ml (glass bottle)
G	22.08.15/22.02.17	1 L
H	-/12.06.15	1 L

Table 1. Packaging information of the examined apple juices.

From the information was given in the table in which shelf life of apple juices were written in the range of between 12 to 18 months. It was observed that on the number 3th and 8th samples expired date and production date were not observed or unable to read as shown in Table 1. Discussing the findings in each apple juice was considered separately and the evaluation was made according to the provisions of the applicable legislation or standards. Analysis were done two times for each product, the following analyzes were performed on the samples.

The pH value for the hydrogen ion concentration; PH meter (WTW pH Inolab 720 series) were determined.

Determination of soluble solid content; The ratio of water soluble dry matter of samples was determined after Refractometer (Leica Reichert Abbe Mark II) device which was calibrated the sample with distilled water.

Hydroxymethyl furfural determination; HPLC method was performed by International Federation of Fruit Juice Producers 1996 [9]. Amount of HMF was determined with the aid of reversed phase

liquid chromatography on RP-18 column by using mobile phase water / methanol and was separated with the help of UV detector.

100 mg to 0.1 mg precision standard HMF were taken in to 100 ml flask and was completed in line with distilled water. For this investigation 10 mg / mL, 15 mg / L, 20 mg / L and 30 mg / L standard solutions for four different concentrations were estimated respectively. Solutions of different concentrations were injected separately in duplicate by using of the 0.45 µm filter paper through via syringe into the device. Calibration curve were created according to area output from the chromatogram which was obtained against each concentration. Apple juice sample was prepared from 25 ml each boxes and transferred into 50 ml volumetric flask then 1 ml Carez I, and then 1 ml Carez II solution were added into sample respectively [9]. The mixture were completed with distilled water up to 50 ml and filtered through coarse filter paper. 2 ml filtrate solution were diluted to 1:1 with water and then the diluted sample were filtered through 0.45 µm filter paper. 20 µm final filtrate solution were injected into the RP-18 column for HMF analysis.

RESULTS and DISCUSSION

PH value of apple juice samples analyzed were estimates between 2.88-3.83 the differences may be due to the cell sturcture of the apple varieties. Water soluble dry matter (WSDM) were found between 11.25% and 11.90% which were specified with Food Regulations of Turkey as shown in table 2.

Sample	PH	WSDM(%)
A	3.40	11.70
B	3.72	11.45
C	3.33	11.25
D	3.36	11.60
E	3.50	11.75
F	3.58	11.90
G	2.88	11.40
H	3.83	11.55

Table 2. The pH and water soluble dry matter values of the apple juice samples.

HMF in apple juice sample that expressed to standart of HMF can be written;

$$\text{HMF} = (\text{AS}_A / \text{AS}_S) \times C_1 \times \text{Ratio of dilution}$$

Where AS_A is the peak area of HMF of the apple juice sample solution, AS_S is the peak area of the HMF standart solution and C_1 is

concentration of HMF standart solution as shown in Fig.2.

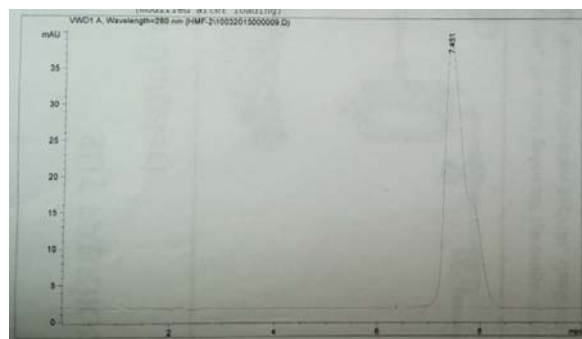


Fig. 2. HPLC chromatogram obtained 5-HMF standart (10 mg L⁻¹).

The HMF for all samples ranged from 1.77 to 7.73 (ppm) 10 mg L⁻¹ depending on the type of companies product and varities of apples as shown in Table 3. These values are comparable to the TS 3633 which was given as up to 10 mg L⁻¹ in which the amount of HMF present in apple juice samples were shown that there is no a significant different. This indicated that all samples were processed in good condition during concentration and filling .

Sam ple	The numb er of worki ng examp les	1	2	Avera ge value	Stand ard deviati on
A	2	1.4 9	2.0 6	1.77	0.16
B	2	5.6 9	5.4 8	5.58	0.02
C	2	5.3 7	4.2 7	4.82	0.60
D	2	2.2 0	2.3 0	2.25	0.01
E	2	3.2 0	3.6 2	3.41	0.08
F	2	8.5 3	6.9 4	7.73	1.26
G	2	5.7 4	6.5 6	6.15	0.34
H	2	2.4 2	2.1 3	2.27	0.04

Table 3. HMF amounts of apple juice samples.

These HMF are resonable agreement with the date presented by several other authers such as Telatar, during the processing of different varieties of apples and apple juice

concentrate in work related to the formation of HMF, the HMF amount were found between 0-1.05 mg/L. The differences may be due to method and varities of applels investigation which were studied on the formation of HMF in the process of apple juice and during the process of evaporation of apple juice at three different temperature. Apple juices were extracted from Amasya, Golden Delicious and Hüryemez varieties which were largely used as raw material in the fruit juice industry. In his work, variety of apple and evaporation temperature were the efficient parameters on the formation of HMF. The HMF content of Hüryemez variety was found to be the most while Amasya variety was found to be the least. As a result, Amasya variety may be suggested as the most convenient one of fruit juice production [6].

Tüfekçi and Fenercioğlu, in their research, the conformities of some of the fruit juices (apple, pomegranate, orange and grape) sold in market to the Turkish Food Regulations were investigeted. The amount of HMF at work in apple juices were found between 1.62-7.49 mg/L. These HMF value are in agreement with our results while HMF of Pomegranate juice and grape juice samples

were above the limit of HMF value . This situation associated with was heat treated at a high temperature of this fruit juice concentrate or was stored in long been inappropriate temperature [10].

Elmastaş, in his study were determined amounts of HMF which forms during processing and storage of foods. The HMF amounts of apple juice were found between 1.01-2.70 mg/L. This result associated with a good production process and shorter duration of materials consumption of apple juices [11].

Lee et al., in their study, improved method for the simultaneous determination of patulin and 5-hydroxymethylfurfural (5-HMF) in sold fruit juices on the local market is described. The amount of HMF at work in apple juices were found between 0.08-14.5 mg / L. In this result, 5-HMF occurred in high incidence (100%) and 19% out of these samples exceeded the IFFJP (International Federation of Fruit Juice Producers) limits [4].

Results show that, the good precision and satisfactory recoveries with less generated makes this method useful for routine quality

control of food products. This study is useful and provides the understanding of HMF levels in various apple juices.

CONCLUSION

In this study, samples of apple juice sold in the market was seen to vary the amount of HMF, PH and water soluble dry matter. Hydroxymethylfurfural (HMF) content of food is an important factor which should be taken into account in production. Simple, a precise and sensitive HPLC method which requires less chemicals and time for the determination of HMF. The method found useful for routine quality control of apple juice. Apple juices have emerged as a result of maintaining the proper storage temperature conditions as a result of the value of HMF content of less than maximum value of 10 mg/L. It is thought that the sensitivity achieved appears as an advantage particularly for the analysis of HMF in apple juices in which HMF concentration is relatively low. Also, well done production stage of the products and keep the short expiry date of the product consumption causes does not exceed the amount of HMF.

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