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Research Article

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Effect of storage temperature on Hydroxymethylfurfural contents in apple juice

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Abstract Hydroxymethylfurfural (HMF) is formed in foods which especially contain sugar if stored at improper temperatures or incurred heat treatment at high temperatures during production. For this purpose, in the markets of İstanbul, samples of apple juice from three different companies were randomly collected for the analysis of hydroxylmethylfurfural (HMF) in apple juice. HMF of the each sample was analyzed by using High Performance Liquid Chromatography (HPLC) in order to determine the effect of the storage temperature at 15°, 25°, 35°C and 2,4,6 weeks' time interval outcome were estimated for three different types of apple juice. During storage the amount of HMF content in the apple juice was increased in relation to storage time and temperature in the range of 4.39-15.89, 1.86-8.24 and 28.55-45.89 mg/L for X,Y,Z types of apple juice respectively. By using of Arrhenius equations, activation energy for each apple juice was estimated between 153.17- 46.48 kj/mol. The effect of temperature and time-tolerance on the increasing rate of HMF in apple juice was explained by Q10 (reaction quotient) values which resulted in the range of 1.07 to 10.60.

Keywords Apple juice; Hydroxymethylfurfural (HMF); HPLC; Activation energy; Q_{10} value.

1. Introduction

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

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]. This chemical conversion has been reported to occur during the storage of fruit juices where the concentration of HMF increases as the storage temperature is increased. The presence of HMF in stored apple juice has also been reported by Burdurlu & Karadeniz, 2003 [3].

HMF consists of aromatic alcohol, aromatic aldehyde and furan ring. Molecular weight of HMF 126.11 g/mol, density 1.29 g/cm^3 in the form of the chemical formula $C_6O_6H_3$. HMF is an intermediate product of well-known Millard Reaction or is formed as a result of dehydration of hexoses under acidic environments [4-5]. HMF is used as an index for formation of juice, milk, honey, cereals, the determination of storage time in many products such as jams, and to understand appropriate chemical heat treatment as shown in Fig.1 [4-6].

HMF is not found in fresh fruits, but it is naturally generated in sugar-containing food during heat-treatment such as drying or cooking. The identification of toxicological relevance of 5-HMF is not clearly documented. Nevertheless, 5-HMF in foodstuff draws more interest because it exhibits mutagenic and DNA strand-breaking activity [7-8].

Besides cytotoxic, nephrotoxic, carcinogenic, genotoxic are among others in vitro activities attributed to 5-HMF [8-10].

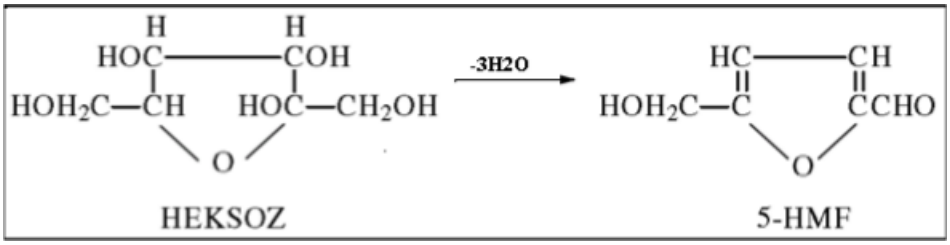

Figure 1: Heating results in the acidic environment of 5-HMF formation of hexoses [5, 11].

Maillard reaction may occur during food processing or storage particularly at high temperatures in carbohydrates and lysine-rich proteins foods. Sugar and lysine are the main compounds involved in the initial states of the Maillard reaction. In advanced states of Maillard reaction, the undesirable compounds such as furfurals can be found [12-13]. If there is a level above a certain amount of HMF, the color browns and this causes the significant deterioration in terms of taste and odor which leads to a reduction in nutrient levels of the product [14]. Marketing the product can partially or completely be eliminated. Therefore, the allowed amount of HMF in foods is limited. Apple juice is indicated by the maximum concentration of 10 mg /L HMF [15].

HMF can be anlysed by different methods such as spectrophotometric, high pressure liquid chromatography (HPLC) and the micellar capillary electro - chromatography (MECC) methods. HPLC method is widely used in HMF analysis. The reason is that it is performed more quickly and easily than other methods. It is also an inexpensive method. For example, it is not necessary to make a big preparation. Only sample dilution and filtration are needed [16].

The aim of this research is to investigate the effect of storage temperature at 15,25, 35°C on the amount of HMF changes during storage of 2,4,6 weeks' time interval and to determine the activation energy and reaction quitent (Q_{10}) values in three types of apple juice.

2. Materials and Methods

Three 100% apple juice samples were collected and randomly selected from same serial products and the code number of the important companies in the Turkish market. The samples were carried to the laboratory in cold chain at 5°C and the initial content of hydroxymethylfurfural was determined for each sample by HPLC method according to the International Federation of Fruit Juice Producers [17]. In this method, the amount of HMF was determined with the aid of reversed phase liquid chromatography on RP-18 column by using mobile phase water / methanol and UV detector at 280 nm. Then the apple juice samples were stored at 15°, 25° and 35°C for 2,4,6' weeks intervals. The specimen information about three types of apple juice was examined and one type of apple juice was indicated as Amasya variety of apple juice. The information about the types of apple juice was shown in Table 1.

Table 1: Packaging information of the examined apple juices.

Product code	Production and Expiration date	Amount of product	The composition of product
X	29.06.15-29.06.16	200 ml	100% apple juice. water, citric acid.
Y	18.09.15-18.09.16	200 ml	100% apple juice of Amasya variety, water, citric acid.
Z	22.08.15-22.02.17	200 ml	100% apple juice. water, citric acid.

The information was given for the shelf life of apple juice as 18 months. The discussion of the findings in each apple juice was considered separately and evaluated according to the provisions of the applicable legislation and standards. The analysis was carried out for each sample and the results were stated in the following.

100 ± 0.1 mg precision standard HMF (Merck Cas- no: 67-47-0) was weighed in 100 ml flask and completed with triple distilled water in order to estimate calibration curve and the standard solution was diluted to 10 mg / L, 15 mg / L, 20 mg / L and 30 mg / L respectively. The solutions of different concentrations were injected separately by

using the 0.45 µm filter paper through via syringe into the device and the calibration curve was estimated [17], as shown in Fig. 2.

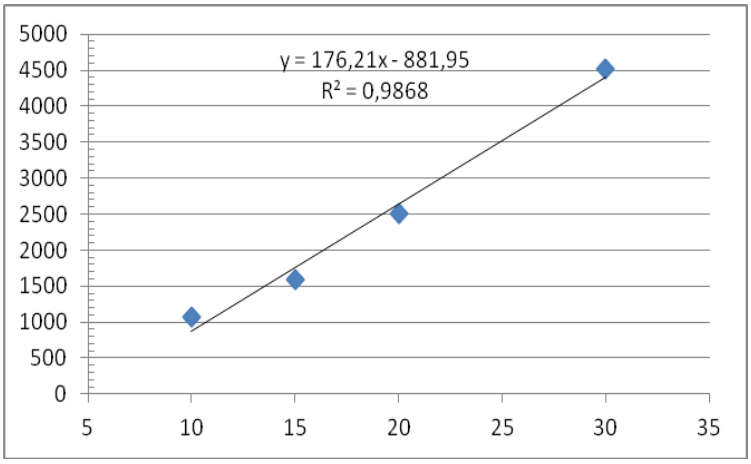

Figure 2: HMF calibration curve

25 ml apple juice from each package was taken and transferred into 50 ml volumetric flask to1 ml Carez I, and then 1 ml Carez II solution was added. The mixture was completed with distilled water up to 50 ml and filtered through coarse filter paper and 2 ml filtrate solution was diluted to 1:1 with triple distilled water then the diluted samples were filtered through 0.45 µm filter paper and 20 µm final filtrate solution was injected into HLPC for HMF analysis [17].

Calculation amount of HMF;

HMF= $(AS_A / AS_s) \times C_1 \times$ Ratio of dilution,

AS_A : the peak area of HMF of the apple juice sample solution,

AS_S : the peak area of the HMF standard solution and

C_1 : concentration of HMF standard solution an example as shown in Fig.3.

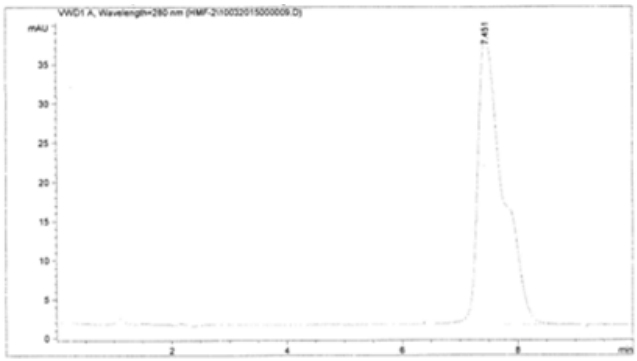

Figure 3: HPLC chromatogram obtained 5-HMF standard (10 mg L^{-1})

3. Results and Discussion

The amounts of HMF for the three types of apple juices at the different storage times and temperatures were shown in Figs. 4, 5 and 6.

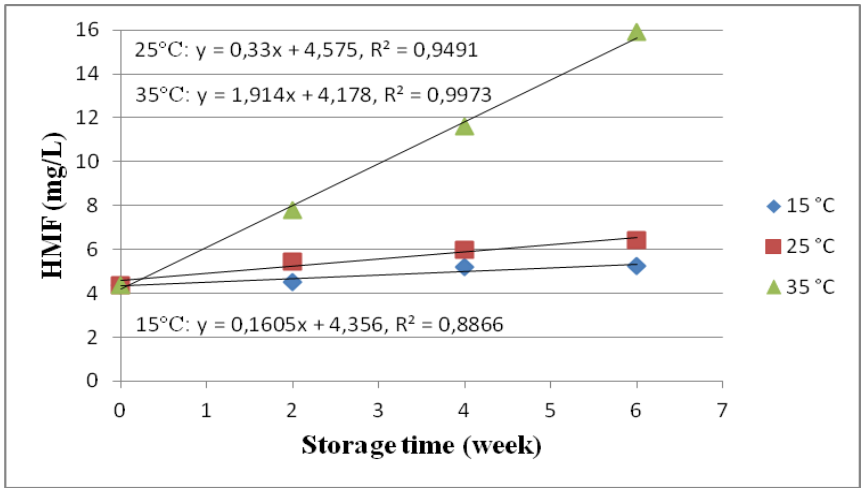

Figure 4: HMF change graphic of X apple juice

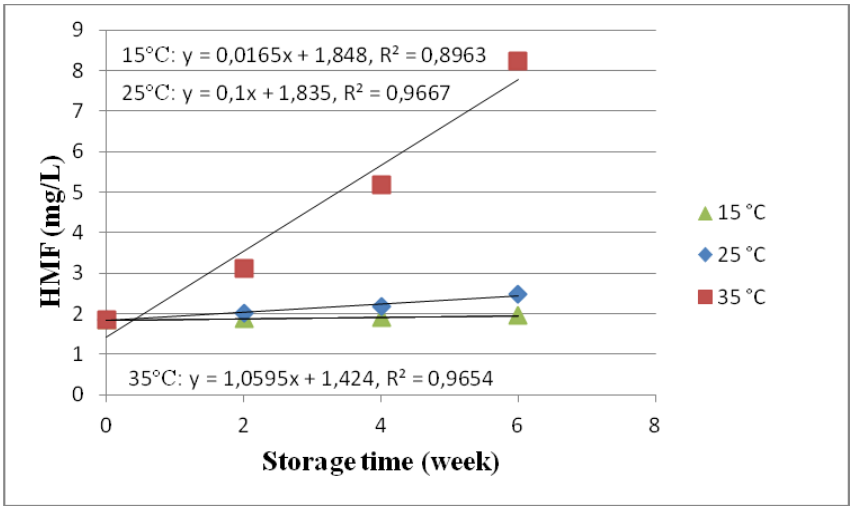

Figure 5: HMF change graphic of Y apple juice

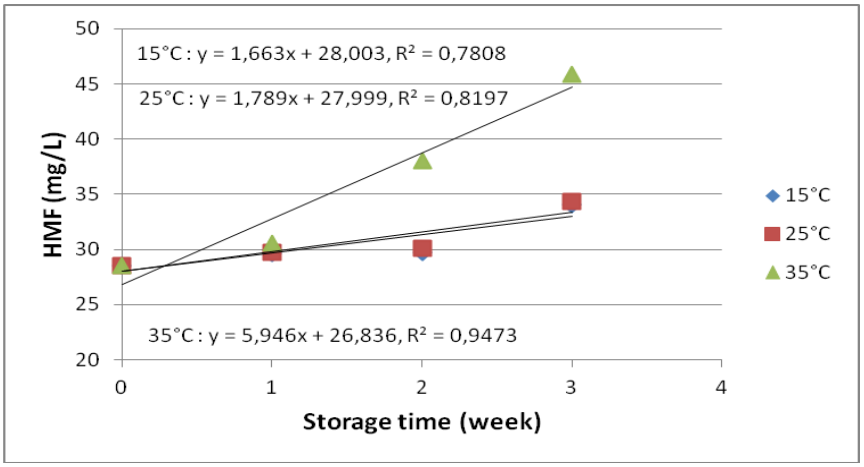

Figure 6: HMF change graphic of Z apple juice

The HMF content in X an apple juice sample initially was estimated as 4.39 mg/L after storage at 15, 25 and 35°C for storage in 6 weeks' time interval and it increased to upper limit of 5.24, 6.41 and 15.89 mg/L respectively as shown in Fig.4. According to these values the amount of HMF did not significantly increase during storage at 15°C and 25°C but high accumulation HMF occurred when stored at 35°C.

Initially 1.86 mg/L content of HMF in Y type of an apple juice (Amasya variety) after storage at 15, 25 and 35°C for 6 weeks' time interval increased to maximum values of 1.96 , 2.48 and 8.24respectively as shown in Fig. 5. The amount of the HMF comparing with X type an apple juice did not increase significantly during storage at the same time and temperature. This may be due to the varieties apple or related with initial content of HMF.

For Z type of apple juice initially contains 28.55 mg/L, which is high in HMF, and after storage at 15, 25 and 35°C for 3 weeks' time interval the amount of HMF increased and reached the highest value of 34.05, 34.38 and 45.89 mg/L respectively as shown in Fig. 6.

The range of HMF value of X apple juice sample at 15°C, 25°C and 35°C in 2 weeks' time can be seen in Fig. 4 in which the values range from 4.39 to 7.81 mg / L. Comparing to these results with AOAC standard specified for apple juice, all the HMF values were estimated in range of 10 mg / L in the standard levels except at 35°C storage temperature. But HMF value of Y apple juice (Amasya variety) samples only changed between 1.86 and 8.24 mg/L for all temperatures as shown in Fig. 5. This is not the same as the findings of the Solomon et al. (1995) [18] who has found that HMF and furfural contents did not significantly change during 52 day storage of orange juice at 8°C the differences may be due to lower storage temperature and type of fruit juice.

When storage time and temperature increased to 35°C for all the samples, the amount of HMF were increased and these results are in agreement with Babsky et al. (1986) [19] who stated that HMF content of apple juice concentrate were increased to 44 mg/100 g after 100 days' storage at 37°C. The HMF concentration at different storage time and temperatures in Golden Delicious apple juice concentrate was found between 0.52 and 96.3 mg/kg, whereas Amasya apple juice concentrate was between 0.52 and 190 mg/kg [3]. Similar results were also reported by Lee and Nagy (1988) [20] as they found that HMF did not increase in canned grapefruit juice stored at 10°C, while high accumulation HMF occurred when stored at 50°C. In this study, the amounts of HMF in X and Z apple juice samples were found to be greater than in Y Amasya apple juice sample. This may be due to more rapid occurrence of the Maillard reaction in X and Z apple juice samples.

3.1. Considering Kinetic Parameters

Temperature dependence of the Maillard reaction was modelled with the Arrhenius equation [21]:

$k = k_0 . e^{-Ea / RT}$

where R is gas constant and Ea is activation energy for reaction rate. The activation energy was calculated from the slope curve for each type of the apple as shown in Figs.7,8,9 and Table 2. The activation energy was found by linear regression analysis to be between 11.11 to 36.61 kcal/gmol, with an R2 value of 0.99 to 0.77. The activation energy activation energy of $X \geq Y \geq Z$ which means that HMF were occurred faster in the Z type than Y and X type of apple juice as shown in Table 2. This may be due to the structure differences between apple juice varieties.

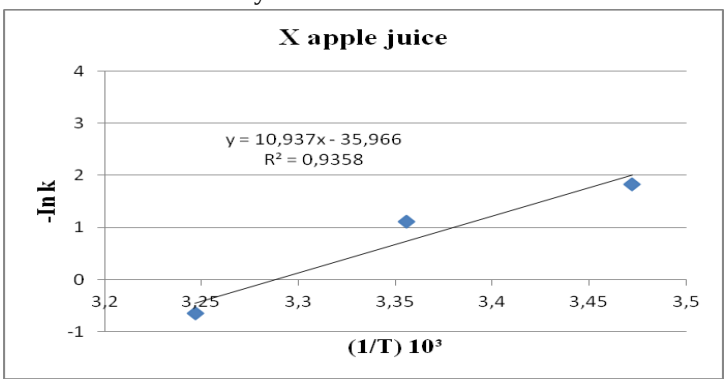

Figure 7: Arrhenius plots of X apple juices at different storage times and temperatures

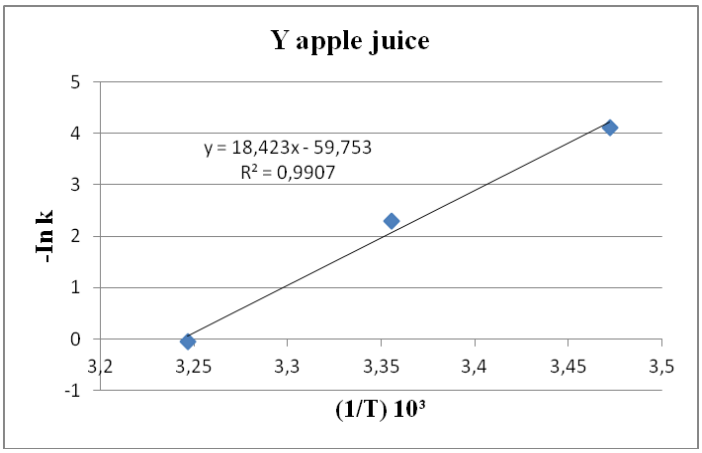

Figure 8: Arrhenius plots of Y apple juices at different storage times and temperatures

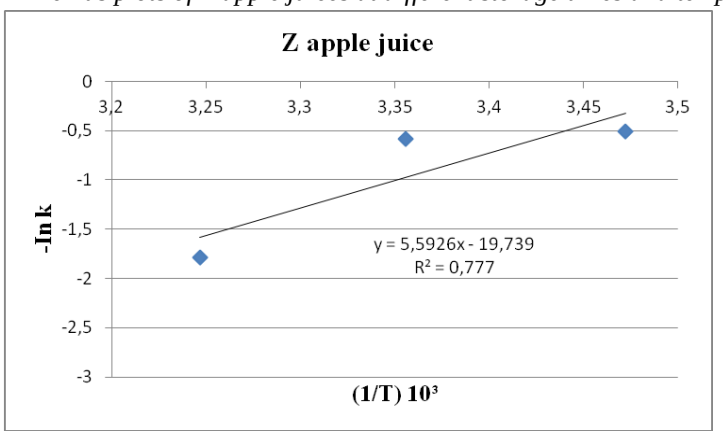

Figure 9: Arrhenius plots of Z apple juices at different storage times and temperatures

Table 2: The kinetic data for the Arrhenius plots of three different apple juices.

Sample	Temperature (°C)	(K)	k (mol/week)	-In k	(1/T) × 10^3 (K^{-1})
	15	288	0.1605	1.8295	3.4722
X	25	298	0.3300	1.1087	3.3557
	35	308	1.9140	-0.6492	3.2467
	15	288	0.0165	4.1044	3.4722
Y	25	298	0.1000	2.3026	3.3557
	35	308	1.0595	-0.0578	3.2467
	15	288	1.6630	-0.5086	3.4722
Z	25	298	1.7890	-0.5816	3.3557
	35	308	5.9460	-1.7827	3.2467

Although the Maillard reaction occurs between reducing sugars and amino acids, the initial rate of this reaction depends on many factors such as material composition, the type of amino acid which were affected on the Maillard reaction browning. Therefore, increasing sugar and amino acids in Y type of Amasya apple juice might have less reactivity in the Maillard reaction than those in other varieties of apple juice.

Q_{10} value expresses that the reaction dependence of temperature which was calculated with the help of the following formula [22]:

$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)}$

where T_1, T_2 = reaction temperature in °K; k_1 = rate constant at T_1 temperature; k_2 = rate constant at T_2 temperature.

Activation energies over the temperature range 15-35°C for three apple juice samples were calculated from the slopes of Arrhenius plots shown in Table 3. Activation energies of X, Y and Z at 15-35°C were found to be 21.73 kcal/mol, 36.61 kcal/mol and 11.11 kcal/mol respectively. The activation energies indicate that browning reactions are in that range of 11.11 ≥ 21.73 ≥ 36.61, which is faster for Z type and lower for Y type of apple juice. The activation energies determined in this study were the same as with other studies such as the activation energies for apple juice with heat treatment at 5-37°C and 37-130°C which was found to be 19.3 and 27 kcal/mol, respectively [23]. In the other study, activation energies for apple juice with heat treatment at 5-67°C for Amasya variety and 5-37°C for Golden variety were found to be 33 and 21 kcal/mol respectively [3, 24]. In their study they determined activation energies for apple juice were between 79.5 kj/mol (19 kcal/mol) and 105 kj/mol (25 kcal/mol).

Table 3: The kinetic datas for HMF of different apple juices at different temperatures.

Sample	E_a		Q_{10}	
	kcal/mol	kj/mol	15-25°C	25-35°C
X	21.73	90.92	2.06	5.80
Y	36.61	153.17	6.06	10.60
Z	11.11	46.48	1.07	3.32

Q_{10} values for X,Y and Z apple samples was estimated, 2.06-5.80, 6.06-10.60 and 1.07-3.32 respectively as shown in Table 3. This indicated the reaction rate for every 10°C change occured in X,Y and Z types for the temperature range between 15-25 °C to 25-35°C and the reaction rate was 2.9, 1.75 and this was 3.1 times faster for the first to the second 10°C changes respectively. These results can be explained that the reaction Q10 values were in agreement with the data presented for activation energy and the amount of the HMF increased during storage.

4. Conclusions

This study also showed that at the storage temperature of 25° and 35°C, HMF levels of all apple juice samples significantly increased at the end of storage time and this increase could especially be seen at the fourth and the sixth weeks (for Z apple juice at the third and the fifth weeks). A significant increase of HMF was observed at the end of storage time at 35°C for each sample. The main contribution of this paper is a kinetic parameters of the non-enzymatic browning of some apple juices under heating conditions. The processing temperature had a strong effect on browning kinetics and amount of HMF for apple juices. Moreover, the applied modification of Arrhenius equation for the dependence of browning reaction rate on temperature was suitable for the prediction of the rate constant providing values which were close to the observed reaction rates in independent experiments.

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