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Kinetics of Nonenzymatic Browning Reactions in Pumpkin Puree During Storage

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Abstract

Pumpkins (*Cucurbita moschata*) are a great source of essential nutrients counting vitamins, minerals, carotenoids, and dietary fiber. Due to their healthy composition, it draws consumer attention. Pumpkin puree is used for preparation of baby foods, soups, flour, jam, jellies, and desserts. To assess the main quality parameters of the foods such as color and hydroxymethylfurfural (HMF) formation, CIE-L*a*b* color changes and HMF formation which is also an indicator of Maillard browning reactions were evaluated storing the pumpkin puree at 27°, 37° and 47°C for 17 weeks. Kinetic parameters for HMF formation and color changes were calculated. The findings showed that HMF quantity linearly increased with the temperature and storage duration following the reaction model of zero order. The values of b* coordinate, Lightness, Chroma, and hue lessened linearly as a* coordinate values increased linearly fitting zero order reaction kinetic. By means of Arrhenius equation, the temperature dependency of the rate constant of color variation was demonstrated and the values of the activation energy (kj mol^{-1}) were calculated as 76.15, 30.60, 46.08, 28.44, 27.61 and 38.32 for HMF formation, Lightness, a* coordinate, b* coordinate, Chroma, and hue, respectively.

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1. INTRODUCTION

Pumpkin, a member of the Cucurbitaceae family covering different species e.g. *Cucurbita moschata, Cucurbita maxima* and *Cucurbita pepo,* is widely grown plant particularly in China, India, Russia, Ukraine, United States of America, Egypt, Mexico, Italy, Spain, and Türkiye. Pumpkin, squash, and gourds production in the world was 23.783.936,41 tons, and harvested area was 1.501.696 ha, while Türkiye's production was 771.651,00 tons, and harvested area was 100.853 ha in the year of 2021 (Kulczynski et al., 2020; FAOSTAT, 2023).

Carotenoids, including lutein and β-carotene, as well as minerals, and vitamins C, B6, thiamine, K, and riboflavin are abundant in this orange-colored and fibrous vegetable. Pumpkin also contains a lot of dietary polyphenols, which have been exemplified to have neuroprotective, chemo preventive, antioxidant, and anti-inflammatory features. Thus, it is considered as a healthy nutrient for adults and children and may be consumed directly or used in a variety of meal preparations. Several types of phytochemicals, involving phenolics, carotenoids, tocopherols, phytosterols, cucurbitacin etc. were detected in the examination of pumpkin fractions. The seeds, peel, and flesh comprise of different ingredients which play an active role as antioxidant and antimicrobial agents in a therapeutic way (Hussain et al., 2022). There are many different countries that benefit from pumpkin as medicine in China, Argentina, Yugoslavia, Mexico, India, America, and Brazil given that its high nutritional value and easing capability of hypertension, diabetes, and liver diseases' symptoms (Kulczynski et al., 2020). Adams et al. (2011) stated that pumpkins also have hypoglycemic impacts.

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Furthermore, some related studies have demonstrated that *Cucurbita moschata* is potentially able to encourage the effects of anti-obesity, anti-diabetic, anti-cancer, and antibacterial (Men et al**.**, 2021).

For the last years, there has been much research conducted on pumpkins and pumpkin based products, such as oil content and composition of pumpkin seeds (Fedko et al., 2020), pumpkin based probiotic beverage (Koh et al., 2018), air drying and kinetic behavior of pumpkins (Guine et al., 2012). Effects of butternut fibres acquired from *C. moschata* on bread production was studied and reported that adding fiber ranging from 5 to 15 grams to 1 kilogram wheat flour can be promising to enhance the structure of the bread following baking process and through storage (de Escalada Pla et al**.**, 2013). Aziz et al. (2023) stated that pumpkin flour might also promote the way of gluten working in the dough such as assisting the rise of the bread and stabilization of the gas cell texture.

On the other hand, browning is among the greatest quality issues observed throughout food processing and its storage. The formation of browning in processed foods is primarily based on the nonenzymatic browning reactions which includes Maillard reaction, caramelization, and the degradations of pigment and ascorbic acid (Cornwell & Wrolstad, 1981). Reducing sugars and α-amino groups are responsible for the first stage of the Maillard reaction (Buedo et al., 2000), which is unfavorable for concentrated or dried foods (Burdurlu & Karadeniz, 2003; Koca et al., 2007), respectively. HMF, a sign of the intensity of applied heat treatment, is also known to take place through the Maillard reaction (Lee & Nagy, 1988).

As consumers' preferences are shaped with the quality and appearance of the foods, process and storage circumstances of a food product should be necessarily controlled. Many researchers have investigated the kinetics mechanism of chemical browning reactions (Toribio & Lozano, 1984; Beveridge & Harrison, 1984; Burdurlu & Karadeniz, 2003; Özhan et al., 2010). Peroxidases and polyphenol oxidases leading to browning, become inactivated enzymes during heat application, otherwise there are various other nonenzymatic paths that cause to color changes in the food along storage, for example, phenolic compounds and carotenoids degradation. The primary carotenoids obtained from pumpkin e.g. lutein, β-carotene, and α-carotene oversee the pumpkin-based products' color. Therefore, all aspects regarding the color loss of pumpkin based products should be examined because color retention is a significant attributor of product quality during storage and carotenoids are not stable at high temperatures. There are few research available reviewing the manufacture of pumpkin puree and the stability of quality parameters throughout its storage (Provesi et al., 2012).

For this reason, kinetic models of the reactions are required to optimize processing and storage conditions and to estimate optimum shelf life of the pumpkin puree. The kinetic parameters like rate constant, reaction order, and activation energy ensure beneficial data both on the quality changes arising from heat treatment applied (Dutta et al., 2006) and on the shelf-life determination of foods through different storage conditions. This investigation aims to specify the kinetics of nonenzymatic browning occurred in pumpkin puree by measuring both HMF content and the values of CIE L*, a*, b*, Chroma (C), and hue (h) during storage at 27°, 37°, and 47°C and to define the correlation among the data obtained from each parameters analyzed.

2. MATERIAL AND METHOD

2.1. Materials

Pumpkins *(C. moschata)* were supplied by local farmers at Niğde and processed into pumpkin puree at Göknur Gıda A.Ş. fruit and vegetable concentrate production factory. According to the process flow diagram, pumpkins were washed, sliced, deseeded, steamed, crushed, heat treated at 95°C for 3 minutes, passed through 2 consecutive screens that are 1.2 mm and 0.8 mm, respectively.

Citric acid was added to prevent enzymatic browning reactions. Pumpkin puree was filled into pouches and capped hermetically before pasteurization applied at 96°C for 15 minutes. Then, it was cooled until reaching to room temperature by applying cold water with ice. This process is repeated for the preparation of replicate samples. Prepared samples were put into incubators as replicated sets at 27°, 37° and 47°C.

Randomly selected replicate samples were taken on weekly basis from 47°C incubator, on two weeks basis from 37°C incubator, for 3 weeks basis from 27°C incubator and analyzed in respect of HMF amount and CIE L*a*b* color indices for the evaluation of nonenzymatic browning reaction in pumpkin puree. Pumpkin purees were also analyzed for titratable acidity (%, citric acid), total soluble solids, and pH to determine the physicochemical properties of the samples.

2.2. Methods

2.2.1. Chemicals

Merck (Germany) was the supplier for all analytical-grade chemicals utilized in this investigation.

2.2.2. Physical and Chemical Analysis

2.2.2.1. Titratable Acidity, Total Soluble Solids (TSS, Bx), and pH

TSS was analyzed at 20°C using ATAGO refractometer (RX-5000α, Atago Co., Ltd., Japan). The distilled water diluted pumpkin puree samples (1:1, v/v) were subjected to pH analysis at 20°C by a pH meter (Mettler-Toledo GmbH, Switzerland). Titratable acidities were analyzed by potentiometric method using the same pH meter by water dilution of the puree samples as (1:1, v/v) and reported as % anhydrous citric acid. Each sample was measured in triplicate.

2.2.2.2. Color Measurement

Color changes in pumpkin puree were determined with the help of Minolta CR-300 reflectance colorimeter (Osaka, Japan). The calibration of the device was performed on a white colored ceramic reference plate before starting the analysis. A glass cell was used in the filling of pumpkin puree samples, and the samples were directly measured without any other treatment. Lightness (L*), a* and b* coordinates, Chroma (C) and hue (h) values were noted. Three evaluations were conducted for each sample.

2.2.2.3. Hydroxymethylfurfural (HMF) Analysis

The amount of HMF in stored pumpkin purees was quantitatively defined by reference to the procedure expressed by Anonymous (1984), which is associated with the colorimetric reaction occurred among barbituric acid, p-toluidine, and HMF generating a red complex. Since red intensity depends on the HMF quantity, the analysis of HMF was implemented by reading the red color at 550 nm with a Spectrophotometer (UV mini-1240 UV-VIS Spectrophotometer, Schimadzu Corp. Kyoto-Japan).

2.2.2.4. Calculation of Kinetic Parameters

The alterations in HMF and CIE-L*a*b* color parameters of pumpkin puree were analyzed through the standard equation for a reaction model of zero-order that means HMF and product's brown color linearly rises with time, described below:

$$C = kt + C_0$$

where C, the concentration at time t; C_0, the concentration at time zero; k, the zero-order rate constant; t, the storage time (week).

Temperature dependence of HMF occurrence and CIE color indexes (L*, a*, b*, C, h) was defined by Arrhenius equation given below:

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

where Ea, the activation energy (kJ mol^{-1}); k, the rate constant ($week^{-1}$); k_0, the frequency factor ($week^{-1}$); R; the universal gas constant (8.314×10^{-3} kJ mol^{-1} K^{-1}); T, the absolute temperature (°K). Additionally, Q_{10} values were calculated with the below equation:

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

where k_2, the rate constant of the browning reaction at the temperature of T_2 (mg HMF week^{-1} and Lightness, Chroma value, hue value, a* and b* color coordinates); k_1 the rate constant of the browning reaction at the temperature of T_1 (mg HMF week^{-1} and Lightness, Chroma value, hue value, a* and b* color coordinates) (Labuza, 1984).

2.2.3. Statistical Analysis

IBM SPSS Statistics programme (Version 29.0.1.0) has been used to conduct statistical analysis. Correlation test at significance level of 0.01 and 0.05 has been performed by determining the correlations between HMF and CIE color parameters.

3. RESULTS AND DISCUSSION

For nonenzymatic browning of pumpkin puree, changes in color parameters and HMF generation compared to storage time were defined as a function of temperatures (27°, 37°, 47°C). The optimum model was opted considering the determination coefficients (R^2) defined using regression analysis. Rate constants were calculated from best-fit regression equations. Alterations in HMF content, CIE L*, a*, b*, C, h values during storage at the temperature conditions of 27°, 37° and 47°C are demonstrated in Figure 1-6, respectively.

HMF amount present in pumpkin puree showed a rise from the formal value of 3.06 mg L^{-1} to 6.3, 8.9, and 14.76 mg L^{-1} at the termination of the storage period at 27°, 37° and 47°C, respectively. Zhang et al. (2019) and Lee and Nagy (1988) found that HMF amount elevated by time and heating temperature in apple puree and canned grapefruit juices, respectively. The highest HMF increase in pumpkin puree was found as 383% at the storage temperature of 47°C. It was followed by the storage temperature of 37°C with 193% increase. Linearly increased HMF content indicating that HMF occurrence in pumpkin puree is in good agreement with zero-order kinetics which is analogous to the outcomes of the two studies (Burdurlu et al., 2006) and (Lozano, 1991) found in citrus juice concentrates and apple juice model systems, respectively.

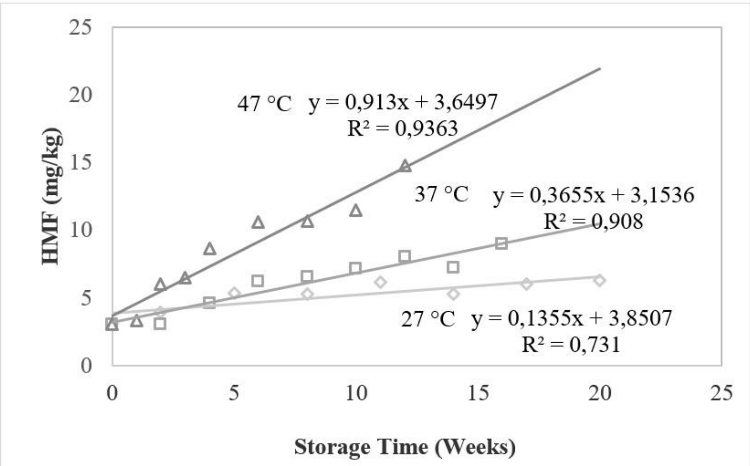

Figure 1. *HMF occurrence in pumpkin puree during storage*

The CIE color indices were considered in the evaluation of browning in pumpkin puree, and it was monitored that while a* coordinate was increasing, L*, b* coordinate, chroma and hue value decreased with time at all storage temperatures (Figure 2-6). Redness increased 32% while yellowness and lightness decreased 24% and 11% at 47°C stored pumpkin puree, respectively. Increases in redness (Figure 3) and decreases in yellowness (Figure 4) and lightness (Figure 2), are the indicators of browning reactions. Hue angle represents the relative amounts of redness and yellowness was also decreased by 8%, 7%, and 5% at 47°, 37° and 27°C, respectively. Özhan et al. (2010) expressed that there was a slight but significant difference in L* value ($p < 0.05$) for carob pekmez samples that were stored at all temperatures throughout the storage period.

Piepiórka -Stepuk et al. (2023) evaluated one of the pumpkin cultivars, namely *Cucurbita moschata,* reviewing the variation in the color indexes such as CIE Lab, C and h values after thermally treating such as blanching, boiling, steaming. According to the results they have, only steaming process have similarities with L* and h values of this research since it was applied same thermal treatment in this study. Provesi et al. (2012) studied on pumpkin purees by keeping the sample at relative humidity and ambient temperature for 6 months. They found that the L*, a*, b* values for *C. moschata* were 41.50 ± 1.06, 7.61 ± 1.09, 27.23 ± 1.74 after storing 120 days, respectively. The results of this study are within similar ranges, but small distinction is likely associated with the preparation of pumpkin puree that is at pilot-plant scale versus industrial scale. Chikpah et al. (2022) reviewed the impacts of varying slice thicknesses, convective air-drying temperatures, and bioactive substances on the kinetics of drying and color change in dried pumpkin slices. Pumpkin's L* and b* indices reduced from the value of 74.61 ± 1.18 to 56.50–70.15 and 61.95 ± 2.03 to 51.90–56.10 kj mol^{-1} respectively while a* value increased from 8.47 ± 0.09 to 9.98-11.07 after drying. During the drying process, the values of L* and b* showed a decline as a* rose. Similar results related with color indices of pumpkin puree were also found in this research.

Dutta et al. (2006) indicated that pumpkin puree darkened as time and temperature rose from 60°C to 100°C through 2 hours and resulted decline in L* value.

Analysis of kinetic data from CIE-L*a*b* color parameters in pumpkin puree revealed that a reaction model of zero order for nonenzymatic browning. Karabacak (2023) produced pumpkin pestils by several drying techniques like microwave, vacuum, and hot air for examining the formation of HMF and color degradation kinetics. L*, a*, b*, C and h values all fitted to zero order reaction model, similar to the kinetic models of this research.

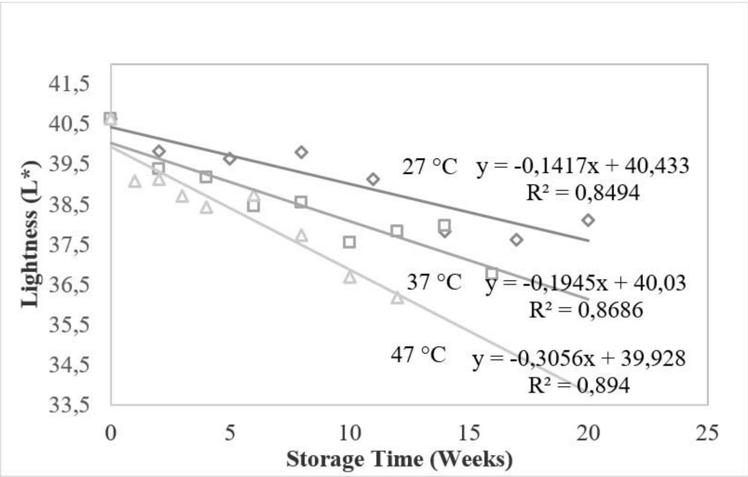

Figure 2. *Lightness changes in pumpkin puree during storage*

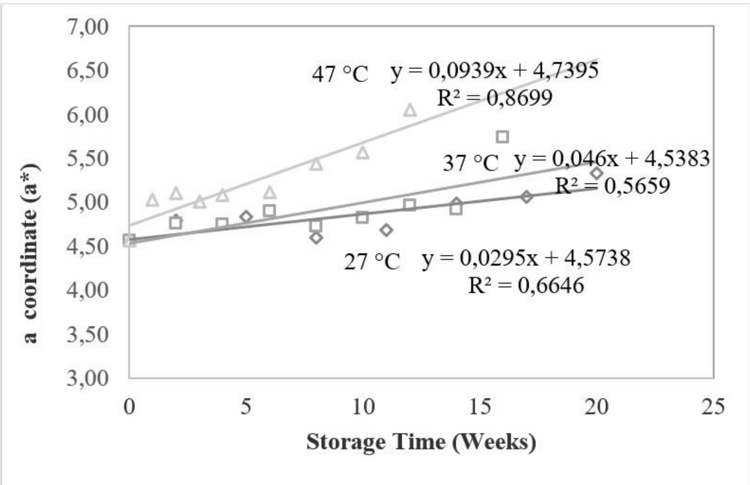

Figure 3. *a* coordinate changes in pumpkin puree during storage*

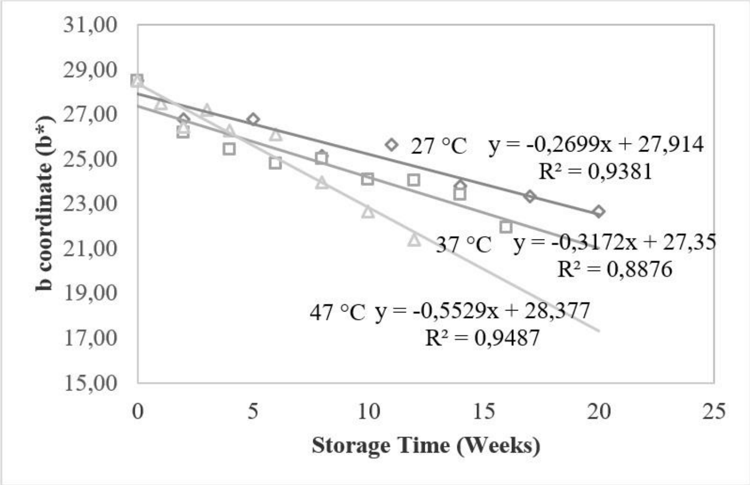

Figure 4. *b* coordinate changes in pumpkin puree during storage*

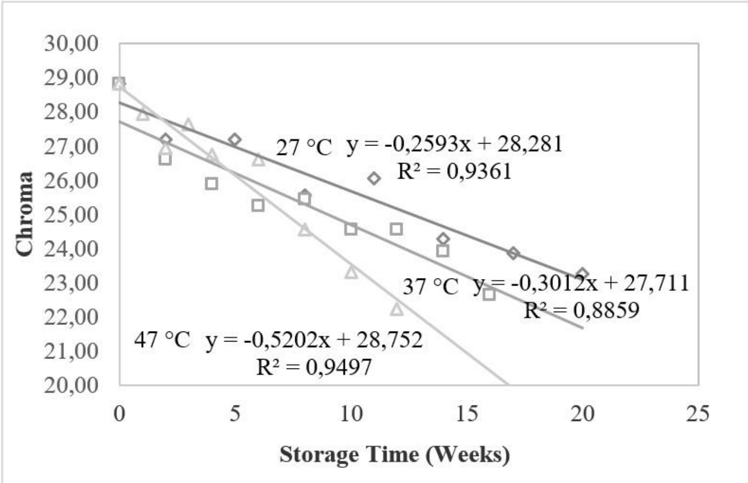

Figure 5. *Chroma changes in pumpkin puree during storage*

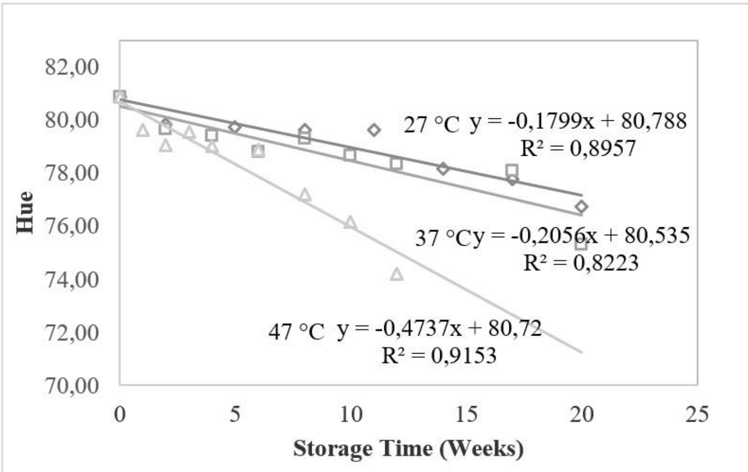

Figure 6. *Hue changes in pumpkin puree during storage*

The heat dependency of the rate constants of HMF generation and L*, a*, b*, C, h values were described by Arrhenius equation (Figure 7). Activation energies for HMF, Lightness, a* coordinate, b* coordinate, chroma and hue values in pumpkin puree in the range of 27° and 47°C were calculated as 76.15, 30.60, 46.08, 28.44, 27.61 and 38.32 kj mol^{-1}, respectively (Table 1).

The value of activation energy present in pineapple juice for the formation of HMF was reported as 29.401 kj mol^{-1} during heat treatment whose temperatures vary from 55°C to 95°C (Rattanathalanerk et al., 2005). The difference in activation energy values might be due to the lower temperature studied in this research and the chemical composition differences between studied food items. In pumpkin puree, higher activation energy needed to initiate the browning reaction. They also explained that HMF and L* value fitted zero order reaction model that coincide with the result of this study.

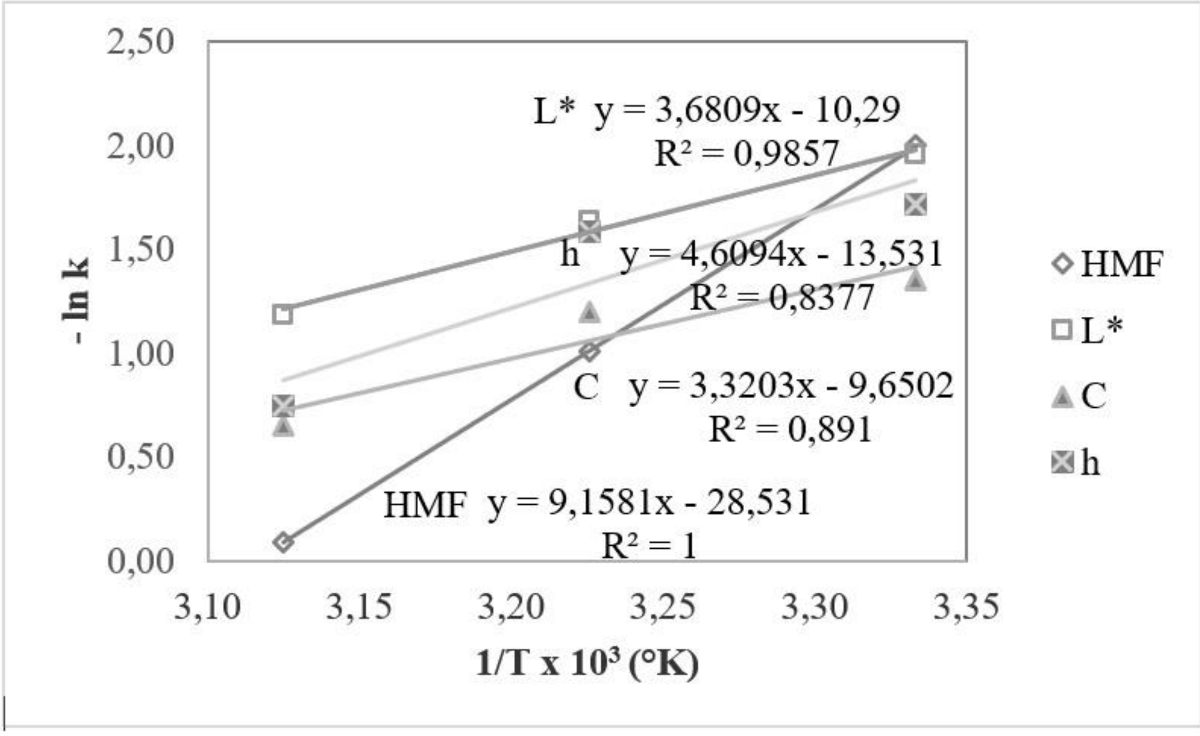

Figure 7. *Arrhenius plots of HMF, L*, C and hue values in pumpkin puree*

The values of Q_{10} obtained for HMF was acquired in the temperature ranges of 27°-37°, 37°-47°C and 27°-47°C were 2.7, 2.5 and 2.6, respectively. The highest Q_{10} value of HMF occurrence detected at the range of 27°-37°C, showing the generation rate of HMF rose about 2.7 times once the temperature increased from 27° to 37°C (Table 1). Burdurlu et al. (2006) clarified that Q_{10} values of citrus juice concentrates for HMF accumulation between 28°-37°C, 28°-45°C and 37°-45°C were much higher than this study's results since citrus products are more susceptible to the storage temperature and time. Additionally, Q_{10} values of color indices (CIE Lab, C, h) were reported between 0.68 and 1.53 for drying process of pumpkin slices, which have similarities with the results of this investigation (Chikpah et al., 2022).

Since both HMF and CIE color parameters all shows browning reactions in pumpkin puree the correlations were evaluated between these parameters and the results were given in Table 2. Except a* coordinate value of pumpkin puree stored at 27°C, a negative high correlation was encountered between Lightness, Chroma, hue, b* coordinate, HMF and C values (r = 0.73-0.94, $p<0.05$), whereas positive correlation was noticed between HMF and a* coordinate (r = 0.72-0.89, $p<0.05$).

At the beginning of the storage TSS, pH, and titratable acidity (%, citric acid), of pumpkin puree were found as 4.98, 4.21 and 0.36, respectively. pH values were found between 4.18-4.21, titratable acidities were found between 0.37-0.41 and TSS were found between 4.60-4.63 in all samples analyzed at the end of storage. This indicates that there are no significant changes in the physicochemical parameters of pumpkin puree stored at varied temperatures and storage periods.

Table 1. *Kinetic parameters for HMF and Color Changes (CIE-L*a*b*, chroma, hue) in pumpkin puree*[a]

Parameters	Storage Temperature (°C)	Zero order reaction equation	Activation Energy ($kJ\ mol^{-1}$)	Q_{10}		
				27-37°C	37-47°C	27-47°C
HMF	27	y = 0.1355x + 3.8507 (0.731)	76.15 (1)	2.70	2.50	2.60
	37	y = 0.3655x + 3.1536 (0.9089)				
	47	y = 0.913x + 3.6497 (0.9363)				
Lightness	27	y = -0.1417x + 40.433 (0.8494)	30.60 (0.9857)	1.37	1.57	1.47
	37	y = -0.1945x + 40.03 (0.8686)				
	47	y = -0.3056x + 39.928 (0.8945)				
a* coordinate	27	y = 0.0295x + 4.5738 (0.6646)	46.08 (0.977)	1.56	2.04	1.78
	37	y = 0.046x + 4.5383 (0.5659)				
	47	y = 0.0939x + 4.7395 (0.8699)				
b* coordinate	27	y = -0.2699x + 27.914 (0.9381)	28.44 (0.8975)	1.18	1.74	1.43
	37	y = -0.3172x + 27.35 (0.8876)				
	47	y = -0.5529x + 28.377 (0.9487)				
Chroma	27	y = -0.2593x + 28.281 (0,9361)	27.61 (0.891)	1.16	1.73	1.42
	37	y = -0.3012x + 27.711 (0.8859)				
	47	y = -0.5202x + 28.752 (0.9497)				
Hue	27	y = -0.1799x + 80.788 (0.8957)	38.32 (0.8377)	1.14	2.30	1.62
	37	y = -0.2056x + 80.535 (0.8223)				
	47	y = -0.4737x + 80.72 (0.9153)				

[a] *Numbers in brackets describe the determination coefficients (R^2)*

Table 2. *Results of the correlation analysis among HMF and CIE-L*a*b* color parameters*[a]

Parameters	HMF		
	27°C	37°C	47°C
Lightness	-0.74*	-0.94**	-0.89**
Chroma	-0.81*	-0.91**	-0.92**
Hue	-0.73*	-0.87**	-0.9**
a* value	0.59	0.72*	0.89**
b* value	-0.81*	-0.91**	-0.92**

[a]*Values of the correlation coefficients for different level of significance *P <0.05 ** P <0.01*

4. CONCLUSION

Hydroxymethylfurfural occurrence and changes in CIE Lab color indexes were quantified to assess nonenzymatic browning reactions observed in stored pumpkin puree at 27°, 37° and 47°C. When the storage period terminated, there was no difference distinguished in pH, titratable acidity, and soluble solids (°Bx) values. HMF formation and all the CIE color parameters followed zero order reaction kinetic. HMF content and L*, b*, C, hue values showed negative correlations (r= -0.74-0.94) for all the storage temperatures studied, while at 37° and 47°C storage temperatures a* coordinate and HMF content showed positive correlations (r=0.72 and 0.89, respectively). The values calculated for the activation energy (kj mol^{-1}) were 76.15; 30.60; 46.08; 28.44; 27.61 and 38.32 for HMF formation, Lightness, a* coordinate, b* coordinate, Chroma, and hue, respectively.

Consequently, CIE Lab values are useful parameters for determination of the degree of nonenzymatic browning reactions occurred in purees without conducting repeated laboratory analysis of HMF content. Thanks to this situation, since it does not only eliminate the labor for analyzing the amount of HMF but also can contribute to the nature by reducing the use of analysis chemicals.

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AUTHOR CONTRIBUTIONS

Methodology and writing-reviewing, F.K., B.I., and S.K.; editing, F.M.; conceptualization and software, O.A., F.M. All authors have read and legally accepted the final version of the article published in the journal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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