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Research article

MODELING THE KINETICS OF HYDROXYMETHYLFURFURAL (HMF) FORMATION IN POMEGRANATE SOUR PRODUCED BY DIFFERENT EVAPORATION METHODS

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ABSTRACT

Pomegranate sour is a traditional functional food that is widely consumed in Türkiye. The aim of this study is to determine the most convenient evaporation method to keep the amount of HMF, a carcinogenic substance formed as a result of high temperatures in the production of pomegranate sour, at low levels. The pomegranate sours were produced using five different evaporation methods (atmospheric pressure (AP), freeze-drying (FD), open air (OA), vacuum assisted (VA) and fan assisted (FA)) and the amount of HMF formed during the process was determined. Kinetic calculations showed that VA and FA methods followed first-order reaction kinetics at 60, 70 and 80 °C, while OA, FD and AP methods followed zero-order reaction kinetics at 80, 90 and 100 °C. The mean Q_{10} value was 1.0006, while the activation energy (Ea) ranged from 72.56 to 126.38 kJ/mol. It was found that the amount of HMF formed varied according to the method, temperature and production time and that the reaction rates increased with increasing temperature, especially for the OA method. In addition, pH, titratable acidity, invert and total sugar content, color, Brix and browning index were analyzed and a kinetic model including all these factors was developed.

1. Introduction

Punica granatum L. is a plant of the Punicaceae family, originating from the temperate and sub-tropical climates (Sharma *et al*., 2011). Pomegranate fruit is a popular functional food due to its taste, color, physicochemical structure, medicinal properties, nutritional value, ease of use, long shelf life, and health benefits for the consumer (Kandylis *and* Kokkinomagoulos, 2020). The rising demand for pomegranate fruit has resulted in notable progress in farming methods, food technology applications, preservation and logistics. Thus, the production, consumption, and commercial value of this fruit have been increasing every year (Kahramanoglu, 2019). The commercial value of the pomegranate fruit and plant is predicted to expand in the coming years due to its multiple uses, including animal feed, oil, medicine, ink, dye, pectin, tannin,

citric acid and vinegar (Sayğılı *and* Sayğılı, 2022). In addition to these uses, pomegranate fruit can also be processed into frozen and dried arils, canned arils, wine, carbonated soft drinks, confectionery, pomegranate sour, and the seeds can be utilized in the cosmetics and pharmaceutical industries. Studies have shown that the bark, flowers, seeds, and fruit of the pomegranate tree have medicinal uses (Vardin *and* Abbasoglu, 2004). Pomegranate seeds and juice are excellent and open for development and it prevents cancer (Hertog *et al*., 1997). Pomegranate has been described on the basis of scientific evidence as a superfruit with therapeutic, anti-inflammatory and anti-edematous effects against a range of diseases, including colitis, diarrhea, stroke, headaches, osteoarthritis, prostate cancer, HIV and cardiovascular diseases (Schubert *et al*., 1999; Malik *et al*., 2005; Sumner *et al*., 2005; Sadeghi *et al*., 2009). To ensure year-round consumption, various preservation methods such as evaporating, freezing, osmotic dehydration, and pasteurization are used for pomegranate fruit (Bchir *et al*., 2009). However, applied heat treatment method and the duration of application can negatively affect the nutritional composition of food. The most problematic compound formed as a result of heat treatment applied to food products is considered to be HMF *(*Anese *and* Suman, 2013*)*. HMF, either on its own or with its by-products such as sulfoxymethylfurfural and chloromethylfurfural, can cause toxicological, mutagenic, and genotoxic effects (Van Putten *et al*., 2013). In addition to its toxic effects at higher concentrations, HMF has been documented to cause damage to the skin, eyes, upper respiratory tract, and mucous membranes. Furthermore, it has been shown to inhibit cell growth and contribute to tumour formation and development (Yangılar, 2013; Batu *et al*., 2014). The level of HMF increases when carbohydrate-rich products undergo heat treatment or are stored for extended periods (Oğuz *et al*., 2023). The presence of increased levels of HMF is indicative of a decline in the quality of the foodstuff in question. This deterioration can be attributed to two main factors: the application of excessive heat during the manufacturing process and the subsequent storage of the product under unsuitable conditions. HMF is used as a heat treatment indicator for in common foods such as milk, coffee, bakery products, grilled meats, chocolate, fruit juices, soft drinks, vinegar, wine, nuts (Shapla *et al*., 2018; Xing *et al*., 2020). Pomegranate sour, obtained by evaporation methods, is a product produced by conventional methods or commercially and consumed as a flavoring sauce in various dishes and salads. As defined by TS 12720 (2016), pomegranate sour is classified as a sour food product. It is produced by pressing pomegranate fruit, clarifying the obtained juice, and thickening it under appropriate conditions. This is then used to flavor foods. The objective of this study is to investigate the effects of different heat treatment methods (vacuum assisted, atmospheric pressure, freeze drying, fan assisted, and open air), as well as temperature and heat treatment time variables, on the formation of HMF in pomegranate sour production and the reaction rate of browning depending on the method and temperature. The study aims to provide data that can be used to address the HMF problem frequently encountered in the pomegranate sour industry.

2. Materials and methods

2.1. Preparation of pomegranate sour

The pomegranate sours have been produced according to five different evaporation methods (Table 1) from pomegranate juice (PJ).

2.1.1. Vacuum assisted evaporation method

To prepare the samples, 1 L of juice of pomegranate (Brix 15.3 ± 0.25%) was placed in a round-bottom flask and concentrated using a vacuum evaporator (Heidolph HEI-VAP Value G1, Schwabach, Germany) at 100 mbar pressure. The concentration process was continued until the water-soluble solids reached a Brix (ºBx) value of 68-72% with ºBx values monitored regularly throughout the process. The dry matter content of the concentrated pomegranate sour was measured using a digital refractometer (HI 96801, Hanna Instruments, Rhode Island, USA). Sours were then produced at three different temperatures: 60 ºC, 70 ºC, and

80 ºC. After preparation, the samples were stored at -18 ºC for further analysis.

Table 1. Applied evaporation methods and their abbreviations.

Evaporation methods	The abbreviations
Atmospheric pressure	AP
Freeze drying	FD
Open air	OA
Vacuum assisted	VA
Fan assisted	FA

2.1.2. Freeze- drying/evaporation

Using this approach, 1 L of pomegranate juice with 15.3±0.25 ºBx% was placed in porcelain containers to produce pomegranate sour. The concentrate was then lyophilized in the device (XO-30F, Nanjing Xianou Instruments, Shanghai, China) at a pressure of 0.1 mbar and a temperature of -65 ºC until the water-soluble solids had decreased to a minimum of 68–72% ºBx. Samples were taken at regular intervals throughout the study to monitor ºBx. A digital refractometer was used to determine the dry matter content of the resulting pomegranate sour. The pomegranate sours produced were stored at -18℃ prior to analysis.

2.1.3. Fan assisted evaporation method

To obtain pomegranate sour, three different glass Petri dishes containing a total of 1000 mL of pomegranate juice with a dry matter value of 15.3% ± 0.25 ºBx were used. The dishes were dried in a fan-assisted cabinet (Elektromag M6040 P, Ankara Türkiye), pomegranate juice was concentrated at three different temperatures (80ºC, 90ºC and 100ºC) until the water-soluble solids reached a minimum of 68-72 ºBx%. Samples were taken periodically to monitor ºBx throughout the study with a digital refractometer and then was stored at -18 ºC for analysis.

2.1.4. Atmospheric pressure evaporation method

To prepare pomegranate sour by this method, 1000 ml of pomegranate juice with 15.3% °Bx dry matter was placed in a stainless-steel container and the water was removed in an open boiler (Gerhardt, EV16, Konigswinter, Germany) by keeping the temperature constant at 95 °C ±2 °C–until the water-soluble solids were at least 68-72% °Bx. ºBx was monitored by periodic sampling during the study using a digital refractometer and the pomegranate sours were stored at -18°C until analysis.

2.1.5. Open air evaporation method

First, 1000 mL of pomegranate juice containing 15.3% ºBx dry matter was placed in a 25 cm diameter glass container with a magnetic stirrer. The water was removed from the mixture using a magnetic stirrer (Heidolph MR Hei-Standard, Heidolph Instruments, Schwabach, Germany) at 500 rpm until the water-soluble solids reached a minimum of 68-72% °Bx. The ºBx value was monitored by taking samples periodically during the study. The dry matter content of the obtained pomegranate sour was quantified using a digital refractometer and the pomegranate sours were stored at -18°C for the experiment.

2.2.Physicochemical analyses of pomegranate sour

In pomegranate sours, acidity (in anhydrous citric acid) % (m/m), water soluble dry matter % (m/m), pH, hydroxymethyl furfural (mg/kg), sucrose % (m/m), glucose % (m/m) and fructose % (m/m), color, browning index (BI) analyses were performed.

2.2.1. Titration Acidity

Titration acidity was determined by titration according to TS 1125 ISO 750 (2002) standard by monitoring pH values and calculated as % (m/m) relative to anhydrous citric acid for all samples. Pomegranate sours were weighed 1.0 g into a beaker and 10 mL of distilled water was added. The solution was

dissolved in a magnetic stirrer and the electrodes of a pH meter (HANNA HI 2002-02 Edge; Hanna Instruments, Germany) were immersed in the solution. Stirring was continued and titration was carried out with 0.1 N sodium hydroxide (NaOH) solution until the pH was 8.3 (TS 1125 ISO 750, 2002).

2.2.2. Water-Soluble Dry Matter

The quantity of water-soluble dry matter in pomegranate sours was determined using a refractometer. A sufficient amount of pomegranate sour was extracted from the sample to be analyzed and placed on the surface of the prism in the sample chamber of the refractometer. The percentage of water-soluble dry matter was then read according to the instructions for use of the instrument (TS 1466/T2, 2011).

2.2.3. pH

pH measurements were performed according to TS 1728 ISO 1842 (2001). The samples to be measured were homogenized by shaking and transferred into a 100 mL beaker. The electrode of the pH meter, which was calibrated using 4 and 7 buffer solutions, was immersed in the homogenized sample and the reading was carried out at 20±2°C (TS 1728 ISO 1842, 2001).

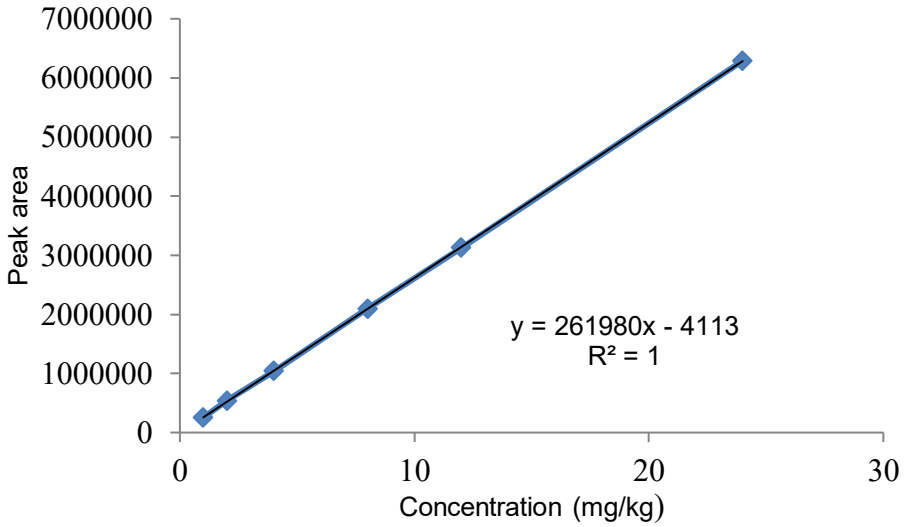

Figure 1. HMF calibration graph

2.2.4. Hydroxymethyl Furfural (HMF)

First, 2.5 g of pomegranate sour sample was weighed into a 100 mL beaker and 12.5 mL of distilled water was added to dissolve it. The prepared solution was transferred to a 25 mL balloon flask and 0.25 mL Carrez I and 0.25 mL Carrez II solutions were added to precipitate the proteins. The prepared sample was filtered through membrane filter paper using a funnel and passed through a 0.45 mm PTFE filter. The filtrate obtained was collected in vials and injected into the HPLC system conditioned with a UV-DAD detector. The HPLC-UV-DAD instrument (Agilent 1100 series, CA, USA) was calibrated with an analytical standard for quantification purposes (Baltacı *and* Akşit, 2016). HMF analysis was carried out according to the 'Harmonised Methods of the International Honey Commission' (Bogdanov *et al*., 2002). The HMF content of each sample was calculated by comparing the peak areas of standards of known concentrations with the peak area in the chromatogram. The chromatographic conditions were as follows:

- Flow rate: 1 ml/min,
- Wavelength: 285 nm,
- İnjection volume: 100 µL,
- Column temperature: room temperature,

- Column type: C18 reversed-phase material chromatography column, 250 mm x 4.6 mm, 5 µm (Nucleosil, USA).
- Isocritical mobile phase: HPLC pure water - methanol (90+10 ml)

Standard solutions were obtained from Merck and contained five different concentrations (0–12 ppm) of 5-hydroxymethyl-2-furfural (HMF) in distilled water. The calibration curve of the standard solutions is shown in Figure 1.

2.2.5. Total sugar content

For the analysis of total sugar, glucose, fructose and sucrose, 2.50 g of the samples were weighed and dissolved in 40 mL of distilled water without heating. The prepared solution was then added to 10 mL of methanol in a balloon jug. The solution was filtered through a filter (PTFE, 25 mm, 0.45 µm), transferred to vials and injected into a conditioned HPLC-RID (Agilent 1100 series, CA, USA) system (Bogdanov *et al*., 2002).

Chromatographic conditions:
- Flow rate: 1.3 mL/min
- Injection volume: 20 µL
- Column temperature: 30 ºC±1 ºC
- Column type: 250x4.6 mm, 5–7 µm diameter modified silica gel column with amine groups (ShodexAsahipak)
- Mobile phase: acetonitrile:water (80:20, v/v)
- Peak and peak area measurements were performed for all standards and samples. A linear calibration graph was constructed showing peak areas or heights in relation to standard concentrations (µg/mL).

2.2.6. Color Analysis

Samples were tested directly without dilution for color analysis. A MINOLTA CR-300 (Minolta Osaka, Japan) color meter was used. Before each measurement, the color measuring instrument was calibrated using white tiles with L* = 97.96, a* = 0.08 and b* = 1.78. The Hunter Lab values were determined from the luminance values L* (light/dark) and the color coordinates a* (red/green) and b* (yellow/blue) (Quek *et al*., 2007). Color measurements were performed in triplicate. The total color difference (ΔE) was calculated (Aslanova *et al*., 2010).

2.2.7. Browning Index (BI)

Firstly, 0.1± 0.001 g of pomegranate sour was dissolved in 50 mL of pure water. The water-soluble pigments were extracted at 25 °C and 140 rpm with axial shaking for 30 minutes, after which the solution was centrifuged (Nüve-Bench Top Centrifuge, NF 1200R, Türkiye) at 8000 rpm for 5 minutes.

The solution was filtered through a 0.45 mm PTFE filter, and the absorbance of the filtrate was read at 420 nm on a spectrophotometer (UV-1800, Shimadzu Corp., 115 VAC, Tokyo, Japan). The absorbance was converted to 0.1 g dry weight to compensate for the moisture content of the samples and the results were calculated (Lee *et al*., 1991; Sarpong *et al*., 2018).

2. 3. Kinetic studies on pomegranate sour

The degree of reaction of HMF formation kinetics in pomegranate sour was calculated using the results of the analyses conducted by Baltacı *et al*. (2016).

2.3.1. Calculation of Reaction Kinetic

To determine the kinetics of the reaction in HMF formed during the processing of pomegranate sour at different temperatures;

a. If the relationship between concentration (C) and time (t) is linear, the reaction is of zero order,

b. If the relationship between concentration in (CHMF/CHMF,0) and time (t) is linear, the reaction is first order,

c. If the relationship between the inverse of the concentration 1/CHMF,0 - 1/CHMF, time (t) is linear (when the initial concentrations are equal), the reaction is second order.

The results obtained were graphed by making the calculations specified in a, b and c and the degree of reaction was calculated (Equation 1, 2) (Baltacı *et al*., 2016).

$$\frac{1}{k}\left(lnC_{HMF} - lnC_{HMF,0}\right) = t \quad (1)$$

$$\frac{1}{k}\left(\frac{1}{C_{HMF,0}} - \frac{1}{C_{HMF}}\right) = t \quad (2)$$

2.3.2. Calculation of activation energy

The temperature dependence of the reaction was determined by calculating the activation energy (Ea) using the Arrhenius equation (Equation 3) (Baltacı *et al*., 2016).

$$k = k_o . e^{-Ea/RT} \quad (3)$$

k: Velocity constant
k_o: Frequency factor
E_a: Activation energy (kJ mol^{-1})
R: Gas constant (8.314x103 kJ mol^{-1} K^{-1})
T: Temperature (K)

For this purpose, the natural logarithms (ln k) of the k values related to the studied reaction were plotted on the Y axis of an arithmetic scale graph and the reciprocal (1/T) of the temperature (K) values corresponding to these values were plotted on the X axis of the same graph and a linear curve was obtained. Regression analysis was applied to this curve and the activation energy was calculated by multiplying the slope of the obtained equation by the gas constant (Baltacı *et al*., 2016).

2.3.3. Calculation of Q_{10} value

The Q_{10} value, which defines the level of temperature dependence of the reaction, was calculated using the equations 4-5 given below (Labuza *and* Schmidl, 1988; Ozkan *and* Cemeroglu, 2002).

$$Q_{10} = 10^{\left(\frac{E_a}{2.303} * R\right) * \left(\frac{10}{T_2 * T_1}\right)} \quad (4)$$

$$\log Q_{10} = \frac{E_a}{T_2 * T_1} * 0.522 \quad (5)$$

T1, T2: Temperatures at which the reaction takes place (K)
E_a: Activation energy (cal mol $^{-1}$ or J mol $^{-1}$)
R: Gas constant (1.987 cal mol $^{-1}$ K^{-1})

2.4. Statistical Analysis

The results of physicochemical analyses of pomegranate sour samples were analyzed using tests such as Duncan test and Principal Component Analysis (PCA) in Addinsoft (2024). XLSTAT statistics and data analysis solution. New York, USA https://www.xlstat.com/en software.

3. Results and discussions

3.1. HMF content of pomegranate sours

The results of HMF analysis using different evaporation methods are given in Table 1–2. The production process with the highest HMF content was AP method. HMF increased from 0.94 mg/kg to 232.84 mg/kg, an increase of approximately 250-fold compared to the initial material. The relatively higher level of HMF formation in this process can be attributed to factors such as the long duration of the process and the excessive temperature rise during heat treatment using a heater. However, HMF formation remained at the lowest level in the FD method (ranged from 0.94 mg/kg to 11.97 mg/kg), this may be due to the low level of sugar degradation caused by low temperature evaporation. The method is effective in minimizing HMF formation, as evidenced by the fact that there was only a 13-fold increase compared to the initial material.

The heat led to an increase in HMF production in both the VA and FA methods. The initial HMF content, which was 0.94 mg/kg in the VA method, was measured at 19.11 mg/kg, 20.65 mg/kg and 23.43 mg/kg at 80 ºC, 90 ºC and 100 ºC, respectively. HMF, which was at the same initial level in the FA process as in the VA process, was determined as 38.99 mg/kg, 42.69 mg/kg, 48.24 mg/kg at 80 ºC, 90 ºC and 100 ºC, temperatures, respectively. The amount of HMF is relatively lower in the VA method, despite the increase in temperature. Vacuum lowers the boiling point, allowing evaporation at lower temperatures and minimizing the production of HMF.

In fact, temperature is the most important factor in controlling how much HMF is formed. A higher temperature degrades the sugars faster and produces more HMF. Bozkurt *et al.* (1999) investigated the formation of HMF and brown pigments in sour and model systems stored at 55, 65 and 75 ºC for 10 days and showed that

temperature plays an important role in the formation of both HMF and brown pigments. On the other hand, HMF in food is studied not only as a useful indicator of thermal processing, but also as an organic compound with potentially harmful properties, making it a food contaminant that has been extensively researched (Kowalski *et al*., 2013).

The results obtained in the study show that one of the most important factors affecting the formation of HMF is temperature and that the heat treatment techniques used in the pomegranate sour production process have a significant effect on this formation. Lower temperatures and carefully controlled heat treatment can be useful in reducing HMF production. The freeze drying method is the most advantageous approach in this situation. Therefore, HMF formation varied significantly among the five different methods used ($p<0.05$). The results indicate that temperature is a critical factor influencing HMF formation during thermal processing by different methods.

3.2. Physicochemical analyses of pomegranate sour

The pH values of the end products produced by all methods decreased compared to the initial raw material pH values (3.10±0.02). The pH values of the end products of the five different methods are rather close and the average is 2.69±0.02. (Table 3).

Table 2. HMF values of different evaporation methods (mg/kg)

Sample Taking	AP	FD	OA	60 ˚C VA	70 ˚C VA	80 ˚C VA	80 ˚C FA	90 ˚C FA	100 ˚C FA
NA0	0.94 ±0.04	0.90 ±0.02	0.94 ±0.04	0.94 ±0.04	0.94 ±0.04	0.94 ±0.04	0.94 ±0.04	0.94 ±0.04	0.94 ±0.04
NA1	3.14 ±0.05	3.00 ±1.80	4.59 ±0.40	1.53 ±0.71	2.70 ±0.32	2.95 ±0.04	10.00 ±1.11	11.41 ±1.62	11.86 ±1.69
NA2	13.75 ±0.67	6.40 ±1.49	6.03 ±0.15	1.45 ±0.57	3.42 ±0.02	4.07 ±1.50	12.13 ±0.69	13.94 ±1.55	15.39 ±1.42
NA3	40.36 ±1.18	7.93 ±0.22	11.27 ±0.53	3.92 ±0.60	4.36 ±0.08	6.17 ±0.93	13.38 ±0.82	15.80 ±1.69	26.13 ±1.01
NA4	77.86 ±1.92	9.28 ±1.16	13.31 ±0.64	8.39 ±0.52	9.42 ±1.52	9.78 ±0.90	15.88 ±0.80	19.86 ±0.85	30.50 ±0.86
NA5	129.96 ±8.36	10.89 ±0.45	18.65 ±3.24	11.44 ±0.15	11.81 ±0.83	13.21 ±0.30	20.08 ±0.76	22.02 ±0.87	36.61 ±1.38
NA6	232.84 ±7.55	11.97 ±0.49	26.32 ±0.66	19.11 ±0.46	20.65 ±3.86	23.43 ±4.74	38.99 ±3.38	42.69 ±4.30	48.24 ±3.56

n=3 parallel studies were conducted. NA is the number of samples taken in each evaporation method until 68±2 ºBx% soluble solids were reached

The highest pH value measured was 2.86±0.01 for the VA method at 80ºC, while the lowest pH value measured was 2.60±0.01 for the FA method at 80ºC and 90ºC. According to the Turkish Standards Institute's TS 12720 (2016) standard for pomegranate sour the pH should be between 2.4 and 4.0. These data are in accordance with the standards (TS 12720) for pomegranate sour.

The differences with other processes may be due to variations in temperature, time, types and amounts of sugars, production techniques or pomegranate varieties used. The evaluation concluded that the thermal treatment applied in the different processes did not significantly affect the pH values of pomegranate sour immediately after production ($p>0.05$).

Kuşs *et al*. (2005) reported that the HMF content in pomegranate sour increased during storage, a feature associated with higher pH values. Another study predicted that lower pH could accelerate the formation of HMF

(İncedayi *et al*., 2010). Kaya *and* Sözer (2005) recorded a pH of 2.05 for pomegranate sour with 71 ºBx produced using the vacuum evaporation method. The pH of pomegranate sour is mainly influenced by the organic acids present in pomegranate juice (Yilmaz *et al.*, 2007). The main organic acid in pomegranate is citric acid, which ranges from 0.2 to 3.2 g/100 mL, with smaller amounts of malic acid (0.9-1.5 mg/mL) and ascorbic acid (0.14-0.69 mg/mL) (Kalaycıoğlu *and* Erim, 2017).

Sugars, together with organic acids, are important components of fruit and have a major influence on taste, contributing to flavor (Famiani *et al*., 2015). The highest glucose content was found in pomegranate sour produced by AP method, 35.84%, while the highest fructose content was observed in FA method at 80ºC, 36.30% (Table ~~2~~3). However, the highest total sugar content was also measured in the pomegranate sour produced by the FA method at 80ºC (70.69%). When the results were examined, the differences in total sugar content between the methods were found to be insignificant. The effect of different temperatures in the VA and FA methods on total sugar content was negligible.

Pomegranate sours have a wide range of total and invert sugar content. This may be due to the water-soluble dry matter content of pomegranate sours and the type of pomegranate fruit used. According to the Turkish Standards Institute TS 12720 standard for pomegranate sour, D-glucose %(m,m) must be at least 20, fructose%(m,m) must be at least 17 and sucrose must not be present (Kowalski *et al*., 2013). All pomegranate sours produced in this study complied with the standards. As a result of the evaluation, the effects of production techniques and temperature applications on total sugar, glucose and fructose levels in pomegranate sour were found to be insignificant ($p>0.05$). Orak (2009) evaluated color, antioxidant activity and some nutritional properties of traditionally evaporated pomegranate juice and concentrate, reporting invert sugar, glucose and fructose values of 46.4%, 23.89% and 22.53% respectively.

Titratable acidity, which is an indicator of the total acid concentration in foods, is an important criterion in the preservation of fruit juices. Titratable acidity affects the unique flavor and aroma of many fruits and fruit juices and is therefore a parameter used to determine overall quality (Turkmen *et al*., 2019). The citric acid content of the pomegranate sours produced by five different methods at three different temperatures was analyzed and found to range from 5.90% to 7.38% (m/m) (Table 3, Figure 2).

The highest titratable acidity was observed in the OA method, with an increase of about six times compared to the initial pomegranate juice, reaching 7.38%. The higher titratable acidity of the OA method compared to the other methods may be due to the increased microbial activity in pomegranate juice processed in the open for a longer period of time. The lowest level of acidity was recorded at 5.90% using the VA method at a temperature of 80ºC. The titratable acidity (g/L) of eight different pomegranate sours produced by the vacuum evaporation method was reported to range from 5.8 to 13.3 in a study by Vardin *et al*. (2008). They also found that the titratable acidity (g/L) of 17 different commercial pomegranate sours ranged from 6.11 to 14.27 (Vardin *et al*., 2008). Oğuz *et al*. (2023) have reported that the titratable acidity of pomegranate sour produced by vacuum assisted and pomegranate sours produced by traditional methods, supplied by sixteen companies, varied between 2.87% and 8.88% as citric acid.

Fruit juice discoloration indicates microbial growth, reducing product quality. Color is a key quality parameter for consumers (Bhat *and* Stamminger, 2015). The L* values, indicating lightness, ranged from 18.58 to 21.06, while the a* values, representing reddish-green, ranged from 8.78 to 16.85 and the b* values, representing blueness-yellowness, ranged from 2.15 to 3.57. The lowest and highest L* values were observed for the VA method at 80ºC and the VA method at 60ºC respectively (Table 3).

These values are similar to those found by Hepsağ *et al*. (2019) and Ergin (2020). The lowest and highest a* values were recorded in the FA method at 90 ºC and the VA at 80 ºC, respectively. The lowest and highest b* values

were analyzed in the FD and the VA at 60ºC, respectively. The greatest color loss was observed in the redness (–a*) value. In the evaluation of the ΔE values of the obtained pomegranate sour in comparison with NA0, all the methods showed color differences that fulfilled the criterion of '5 < ΔE - the analyst observes two different colors'. Therefore, the color difference between NA0 and NA6 was found to be significant for each method ($p<0.05$). Among the different methods, the highest color difference was observed in the AP method, and the color differences between the methods were also found to be significant ($p<0.05$).

The evaluation concluded that the thermal treatment applied in all methods of pomegranate sour production resulted in a darkening of the color data from red to reddish brown (Table 3). Until at least 68% ºBx was reached, there was a noticeable color shift in each technique compared to NA0 (Figure 3).

The data in Table 3 and Figure 4 showed that the highest browning index in pomegranate sour samples prepared by different techniques was 75.86±0.29, which was found in AP method, while the lowest browning level was 44.26±0.57 in FD method. The higher level of browning in AP pomegranate sour compared to the other techniques is attributed to the non-enzymatic browning reactions that occur in products exposed to high temperatures. When the methods were compared, the effects of thermal treatment at different temperatures and durations on the browning index were significant ($p<0.05$).

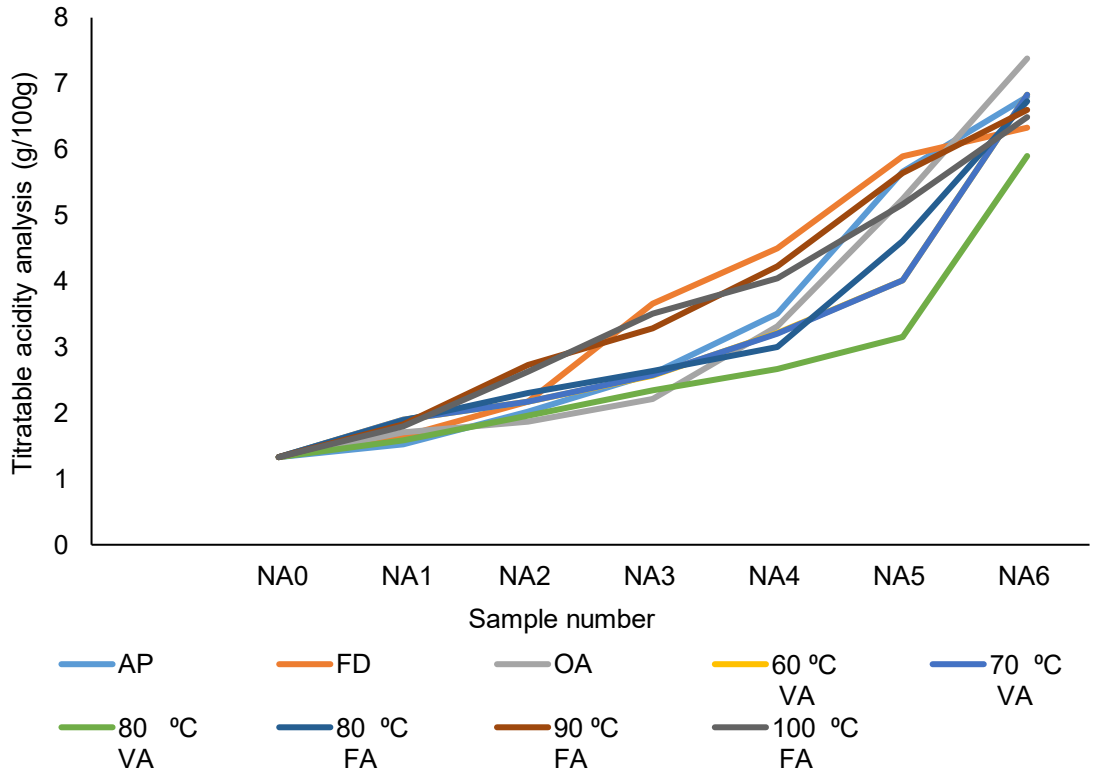

Figure 2. Titratable acidity values of different evaporation methods (g/100g) from the initial product to the end product

Table 3 Physicochemical analysis results of the end product of different evaporation methods

Production Method	HMF mg/kg	pH	% Fructose	% Glucose	% Sucrose	% Total Sugar	% Total Acidity	L*	a*	b*	ΔE*	BI	BI(A_{420})
AP	232.84^{f}* ±7.55	2.65^{a} ±0.01	35.84^{g} ±0.34	31.41^{b} ±0.16	<LOQ	67.25^{b} ±0.18	6.80^{g} ±0.22	20.46^{b} ±0.03	12.36^{f} ±0.16	2.94^{d} ±0.04	13.61^{f} ±0.07	75.86^{h} ±0.29	0.311^{j} ±0.010
FD	11.97^{c} ±0.49	2.64^{a} ±0.00	35.45^{g} ±0.16	32.91^{b} ±1.44	<LOQ	68.36^{b} ±1.28	6.33^{c} ±0.53	19.57^{c} ±0.16	9.47^{b} ±0.07	2.15^{a} ±0.18	8.14^{c} ±0.20	44.26^{b} ±0.57	0.234^{f} ±0.004
OA	26.32^{a} ±0.66	2.64^{a} ±0.01	33.79^{fg} ±1.94	34.66^{c} ±0.84	<LOQ	68.45^{d} ±1.10	7.38^{h} ±0.13	18.65^{a} ±0.32	10.59^{c} ±0.04	2.35^{ab} ±0.10	9.47^{d} ±0.26	51.41^{d} ±0.35	0.286^{i} ±0.002
60 ˚C VA	19.11^{b} ±0.46	2.78^{b} ±0.01	33.74^{b} ±0.32	34.86^{d} ±0.38	<LOQ	68.60^{bc} ±0.71	6.83^{g} ±0.37	21.06^{e} ±0.14	13.57^{d} ±0.02	3.57^{c} ±0.04	9.64^{d} ±0.15	55.73^{e} ±0.36	0.091^{b} ±0.003
70 ˚C VA	20.65^{bc} ±3.86	2.78^{b} ±0.01	34.28^{d} ±0.79	36.06^{e} ±0.61	<LOQ	70.34^{e} ±1.40	6.83^{g} ±0.35	18.90^{f} ±0.09	16.15^{e} ±0.07	3.55^{d} ±0.03	10.36^{e} ±0.07	61.38^{f} ±0.25	0.108^{c} ±0.002
80 ˚C VA	23.43^{bc} ±4.74	2.86^{b} ±0.01	33.65^{c} ±0.20	35.52^{de} ±0.81	<LOQ	69.17^{d} ±0.61	5.90^{b} ±0.14	18.58^{a} ±0.09	16.85^{f} ±1.30	2.57^{b} ±0.22	11.8^{g} ±1.19	72.37^{g} ±3.34	0.118^{d} ±0.006
80 ˚C FA	38.99d ±3.38	2.60^{a} ±0.01	34.38^{d} ±1.03	36.30^{e} ±0.28	<LOQ	70.69^{e} ±0.76	6.73^{f} ±0.01	20.20^{d} ±0.05	8.83^{b} ±0.07	2.90^{c} ±0.16	7.07^{b} ±0.12	45.34^{bc} ±0.65	0.218^{e} ±0.009
90 ˚C FA	42.69de ±4.30	2.60^{a} ±0.01	34.56^{de} ±0.21	33.83^{c} ±1.72	<LOQ	68.39^{cd} ±1.93	6.60^{e} ±0.69	20.26^{de} ±0.05	8.78^{b} ±0.03	2.96^{c} ±0.06	6.98^{b} ±0.06	45.42^{bc} ±0.14	0.245^{g} ±0.001
100 ˚C FA	48.24^{e} ±3.56	2.66^{a} ±0.01	35.06^{ef} ±0.20	33.82^{c} ±0.74	<LOQ	68.88^{cd} ±0.54	6.49^{d} ±0.22	20.93^{f} ±0.17	8.94^{b} ±0.10	3.41^{d} ±0.23	6.54^{b} ±0.21	47.13^{c} ±0.75	0.263^{h} ±0.001

*Different letters in the same column in the table indicate significant statistical difference in pairwise comparison ($p<0.05$). n:3. Duncan test was applied.
**The values of LOQ: % 0.5 for sucrose, 0.15 for glucose, 0.10 for fructose.

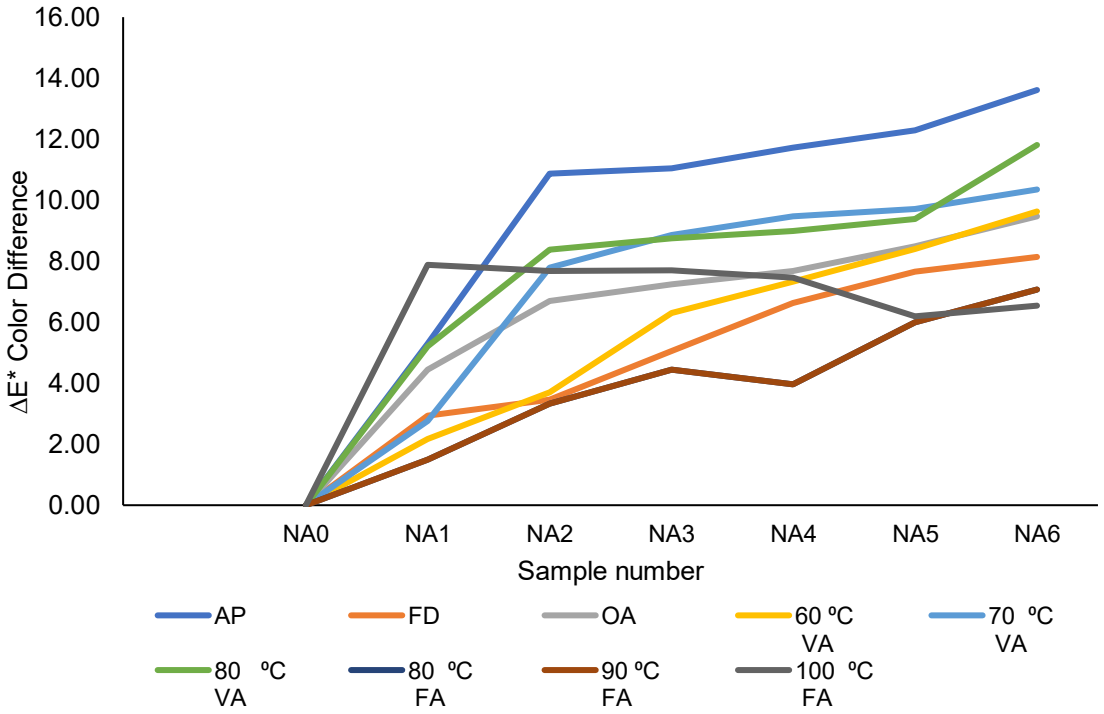

Figure 3. Color differences (ΔE*) from the initial product to the end product of different evaporation methods

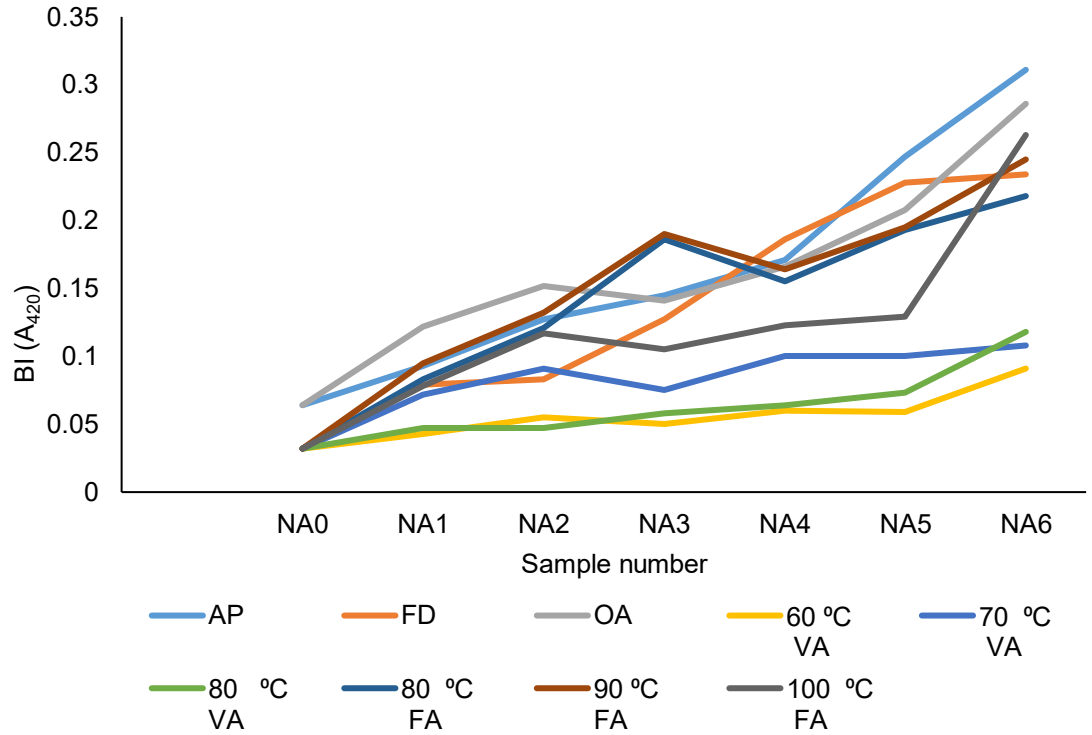

Figure 4. Non-enzymatic browning index values of pomegranate sours produced by different evaporation methods from the initial product to the end product

3.3. Kinetic parameters

Equation ~~6 and 7~~ 1 and 2 were utilized to determine the reaction order of the formation of HMF during the processing of pomegranate sour under different methods and temperatures. If the relationship between concentration (C) and time (t) is linear and the R^2 values are close to 1, the reaction was considered to be of zero-order. If the relationship between the logarithm of the concentration (ln C) and time (t) is linear and the R^2 values are close to 1, the reaction was considered to be first-order. If the relationship between the inverse of the concentration (1/C) and time (t) (at equal initial concentrations) is linear and the R^2 values are close to 1, the reaction was considered to be second-order (Sarpong *et al*., 2018). Degrees of reaction, activation energies, reaction rates, R^2 and curves are given in Table 4.

Table 4 Reaction rate, activation energies and reaction order values

Production method	Reaction Rate (k)(mol/s)	Reaction curve	R^2	Activation Energy E_a (kj mol^{-1})	Reaction Order	Q_{10}
AP	1.60 X 10^{-3}	y = 0.0016x + 0.4185	0.966	119.93	1	-
FD	6.00 X 10^{-5}	y = 6E-05x + 0.0791	0.984	72.56	0	-
OA	4.00 X 10^{-4}	y = 0.0004x – 1.6247	0.981	99.25	0	-
60 °C VA	5.00 X 10^{-4}	y = 0.0005x – 0.1801	0.963	110.29	1	-
70 °C VA	6.00 X 10^{-4}	y = 0.0006x + 0.0816	0.956	113.08	1	-
80 °C VA	1.10 X 10^{-3}	y = 0.0011x - 0.0336	0.981	114.60	1	-
80 °C FA	1.00 X 10^{-3}	y = 0.001x - 0.8188	0.940	121.95	0	-
90 °C FA	1.50 X 10^{-3}	y = 0.0015x - 0.4076	0.928	125.40	0	-
100 °C FA	2.80 X 10^{-3}	y = 0.0028x - 1.5442	0.969	126.38	0	-
					VA 60-70 °C	1.0008
					VA 70-80 °C	1.0007
					FA 80-90 °C	1.0005
					FA 90-100 °C	1.0004

A zero-order reaction is one in which the rate of the reaction remains constant, independent of the concentration of the reactants. If a plot of concentration (c) against time (t) is linear, with an R^2 value close to 1, this demonstrates that the reaction is occurring at a constant rate, with no dependence on concentration. This means that the rate of degradation of a reactant (such as the sugars in pomegranate sour) remains the same over time, regardless of how much reactant is present. In a first-order reaction, the rate of the reaction is directly proportional to the concentration of a reactant. If a plot of the logarithm of the concentration (ln C) versus time (t) is linear, with an R^2 value close to 1, the reaction is first order. As the concentration decreases, so does the rate of reaction. For example, the formation of HMF from sugars may follow a first order rate if the concentration decreases exponentially with time. The rate depends on the square of the concentration of one reactant or the product of two reactant concentrations in a second order reaction. If the plot of the reciprocal of the concentration (1/C) versus time (t) is linear and the R^2 value is close to 1, the reaction is second order. This indicates that the reaction rate is more sensitive to changes in reactant concentration. A decrease in concentration results in a sharp decrease in the reaction rate, which may be true for complex chemical transformations during pomegranate sour processing.

The impact of temperature on HMF formation in different methods was investigated in the study. Temperature is one of the parameters influencing the formation of HMF. The HMF content in pomegranate sour samples showed an increasing trend with increasing temperature. It was observed that the HMF value

of pomegranate sour increased as the processing time increased. The results of this study are consistent with the literature. In the study by Baltacı *et al*. (2016), which investigated the effect of heat treatment on HMF formation in herle (mixture prepared for fruit leather production), it was observed that HMF formation increased as the duration and temperature of the applied heat treatment increased. It has also been reported that storage temperature and duration have a significant effect on HMF formation in samples of sour prepared from grapes, mulberry and carob (Toker *et al*., 2013; Tosun, 2004).

The Q_{10} value is a measure of the rate of increase in reaction rate when the temperature is heightened by 10 ºC. Q_{10} values indicate the degree to which a reaction is affected by temperature changes. A high Q_{10} value for a reaction can be interpreted as meaning that the reaction is very sensitive to temperature changes. The Q_{10} values for the study are shown in Table 4. When examining the table, it was found that the values were around an average of 1.0006. It is therefore evident that the Q_{10} values for the three different temperatures in the vacuum and FA (fan assisted evaporation methods) are very low. This indicates that there is no significant change in reaction rate with a 10 ºC increase in temperature.

3.4. PCA analysis of physicochemical properties

PCA (Principal Component Analysis) is a highly effective method for exploring relationships between variables and observations. This method allows for the simultaneous graphical analysis of data and all variables.

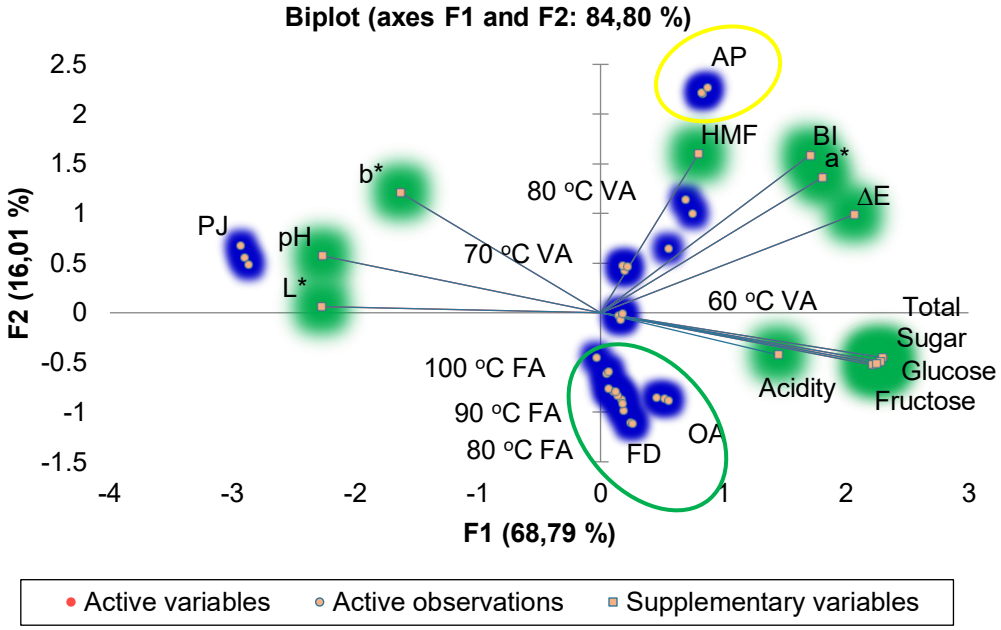

Figure 5. Principal Component Analysis (PCA) score plot of pomegranate sours obtained different evaporation methods

In the PCA analysis of the pomegranate sour samples, PCA1 and PCA2 diagrams explain 84.80% (Figure 5) of the cumulative variance, while PCA5 explains approximately 99.58%. The pomegranate sour samples produced by different methods form four groups: a group in a red circle (PJ), a group in green (FD, OA and FA at 80, 90 and 100°C), a group in yellow (AP) and a group in black (VA at 60, 70 and 80°C). The scores are divided into three ranges according to the physicochemical analyses carried out. The differentiation between the samples indicates variations in the physicochemical parameters studied. The sample in the red group corresponds to the pomegranate juice used in production. Figure 5

shows that the pomegranate juice used differs from the other samples in terms of L*, b* and pH values.

The AP sample in the yellow group stands out from the other samples due to its high HMF content and low L* and b* values and forms a distinct group. In addition, except for pomegranate juice, the analysis results of the samples obtained by the different evaporation methods are similar in terms of acidity, pH, fructose, glucose and total sugars. In the green group, which includes vacuum evaporation, GB and DB methods, the HMF values were relatively lower compared to other methods. In the blue group, the oven evaporating methods resulted in HMF levels close to the limit of 50 mg/kg. The L* value showed a strong negative correlation with the a*, ΔE, BI and BI (A_{420}) values, while it had a strong positive correlation with the b* value. There was a strong negative correlation between acidity and pH. The HMF value showed a strong positive correlation with the a*, ΔE, BI and BI (A_{420}) analyses, but a weak negative correlation with the L* and b* values. According to the PCA analysis, the VA, FD and OA evaporation methods (green and black groups) stand out in pomegranate sour production. While acidity, pH, glucose, fructose and total sugar analyses were similar in all methods, color analyses (L*, a*, b*, ΔE, BI and BI (A_{420})) and HMF levels were higher in methods with more intense heating processes.

4. Conclusion

The formation of HMF and browning resulting from non-enzymatic browning reactions was found to be highly significant during the processing of pomegranate sour using different methods, temperatures and durations. The rate of these reactions increases with increasing temperature, with the highest levels observed in AP evaporation method.

While acidity, pH, glucose, fructose and total sugar analyses showed similar results in all methods, color analysis (L*, a*, b*, ΔE, BI and BI(A_{420})) and HMF levels were higher in the higher temperature methods. Color values were determined using the CIELAB; Lab* color system and a numerical decrease in L* values, an increase in a* values, a decrease in b* values and an increase in ΔE values were observed at different temperatures and evaporation methods. In addition, the decrease in L* values and the increase in ΔE values show significant color changes. Another critical point in the quality production model is the control of temperature parameters and processing time in the production of pomegranate sour. In processes where high heat is applied directly in open boilers and for long periods of time, HMF levels increase significantly. This study concluded that raw material, temperature and time are the main parameters for HMF formation in a quality production model. Therefore, temperature and duration must be carefully controlled and monitored to minimize HMF formation and ensure quality production.

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