INTERNATIONAL JOURNAL OF ELECTRONICS, MECHANICAL AND MECHATRONICS ENGINEERING Vol.7 Num.2 - 2017 (1419-1432)

IJEMME

Received: 28.02.2017
Accepted: 18.03.2017

Removal of Bitterness by Using of Amberlite in Orange Juices

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Abstract - In this study, the removal of bitterness in Washington (navel) orange juice due to limonin was examined by using "Amberlite XAD-7HP" adsorbent. In the meanwhile, the changes on some chemical properties of orange juices such as (pH, titratable acidity, brix) were investigated. It had been determined that the adsorbent was successfully removed the bitterness in Washington navel orange juice. The results showed that bitterness caused from limonin was decreased from 90% to %96 at 30°C and 40°C, respectively. The bitterness of orange juice caused due to limonin was decreased up to %96 during using Amberlite XAD-7HP polymeric resin.

Keywords - *Washington (navel), removal bitterness, limonin, Amberlite XAD-7HP*

1. Introduction

Orange juice should have a typical flavor of mature oranges, orange color and free from any flavor defects. Factors such as color, ascorbic acid content, taste balance (arthritis / acid ratio), bitterness (flavonoids), essential oil content, pulp content and turbidity are important properties in orange juice in terms of the qualities of fruit juice in orange juice [1-4]. The color characteristic in orange juices is one of the most important quality criteria. The dark and natural bright orange color which is formed by carotenoids in fruit juice vials and by the coloring of flavedo is preferred [5-7]. It has been reported as the main carotenoids in the orange juices as α-carotene, β-carotene, γ-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, zeaxanthin, antherxantine and violaxanthin. B-carotene and β-cryptoxanthin are the main pigments that give color to fruit juice and shell color [5,7,8]. Most of the ingredients that provide orange flavors are shell fat-derived and lipid-like, together with many volatile substances, which are predominantly bitterness, sourness and sweetness [9].

The bitterness of fruit juices may be caused by bitter fruit or may be caused by faulty processes during processing [10]. There is a marked bitterness in the Washington (navel) oranges after a couple of hours of squeezing and two different views about the bitterness because of waiting on the juices obtained from Washington (navel) oranges [1,11]. The first is the acceptance of a physical change due to the solubility of the bitter item and the second is a chemical change which causes the fruit to be converted into a bitter compound by squeezing the pre-existing bitter substance. One of the most important examples of physical change is given by the fact that the limonene is poorly soluble and is either heated or kept for a long time to increase the concentration and form a bitter taste.

In chemical exchange, pH has a significant effect. While the precursor substances of the whole fruit do not come into contact with the fruit with acid character, the reaction does not occur, whereas the preliminary substances found in the water contents obtained after squeezing are bitterness as a result of reaction with acid type fruit [11]. Limonin bitterness is perceived in about 4 to 6 mg / L of orange juice, causing an unpleasant strong soreness after 12-15 mg / L, and this bitterness is an important problem in making fruit juice in Washington (navel) oranges [2,12,13]. Numerous researches have been carried out with biochemical, physicochemical, chemical and microbiological methods with the purpose of eliminating the limonin which causes the bitterness in all the citrus juices, especially orange juice presented for the time being [14-23].

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In the present study, the removal of limonin bitterness from Washington navel orange juice by using the Amberlite XAD-7HP resin which is a good adsorbent thanks to its aliphatic structure, was investigated. Amberlite XAD resins have physically or chemically been loaded with the various ligands to prepare new chelating resins and explored extensively for metal enrichment. Their attractive features are easy regeneration for multiple sorption-desorption cycles, good mechanical stability and reproducible sorption characteristics. The impregnation of polymer matrices with common chelating ligands is an easy way to design chelating collectors. Amberlite XAD-2 [24–28], Amberlite XAD-4, Amberlite XAD-7, Amberlite XAD-16 [28] and Amberlite IRC-718 [29] have been used as a support for such preparations. During this process, it was aimed to contribute to the literature by investigating the effects of changing the qualitative properties such as taste, aroma, pH, titration acidity, amount of limonin in orange juice to orange juice.

2. Material and Methods

In this study, the orange samples of "Washington (Navel)" were bought from the market in Istanbul in 2015 citrus season and brought to Istanbul Aydin University Food Engineering - Instrumental Analysis Laboratory. The samples were stored in a refrigerator at (+4)°C until analysis required.

Amberlite XAD-7HP (Sigma-Aldrich, 101503324) resin was used to remove limonin in the orange juice with the appropriate adsorbent, and the effect of this resin on the temperature of the limonin was evaluated at the appropriate temperature. Before the orange juice was obtained, the oranges were washed out after separating from the stalk and leaves and cut into two. The orange juice (super mixer) was used to extract the juice of orange. In order to separate the orange juice from the coarse pulp, a 1 mm plastic sieve was poured with the aim. The resulting orange juices were heat treated at 95°C for 15 seconds (Stuart SWBD, Water Bath) and cooled to room temperature. Limonin, water-soluble dry matter (°Brix), pH and titration acidity analyzes were carried out in the heat-treated orange juices. Orange juice was centrifuged (Hettich, Rotofix 32A) at 4000 rpm for 10 minutes during the removal of bitterness of limonin in orange juice to allow separation of serum and pulp. After centrifugation, 100 ml were taken from the serum and the system was supplied with a flow rate of 0.2 ml / min, adjusted with a peristaltic pump (Aqua TEC1), and at 30°C, 40°C, 50°C and 60°C, from the condenser column (Borucam) with adsorbent, 1 cm in diameter, 30 cm in length and 30 ml in volume. The temperature control in the condenser column was provided with a water bath (Julabo F12). Thus, and the serum part was removed with the help of adsorbent by applying different temperature as shown in Figure 1. The sample of orange juice from the column was taken and passed through the 0.45 μm syringe filters and then 20 μL was taken and injected into HPLC (Agilent Technologies 1200 Series) in order to analyze limonin.

This study which is related with the removal of limonin in the orange juice using the adsorbent the procedure was repeated three times for each applied temperature and the amount of limonin were determined as three parallel for each orange juice sample.

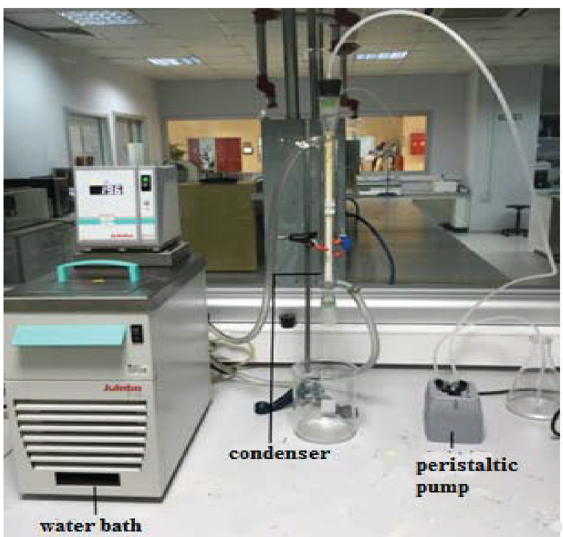

Figure 1. The system installed to eliminate the limonin in orange juice with the adsorbent application

2.1. The Determination of limonin amount

20 µL of the standard limonin (Santa Cruz Biotechnology-1180-71-8) solutions (5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 30 mg/L, 35 mg/L, 40 mg/L) prepared to generate the standard calibration curve were injected into HPLC (Agilent Technologies 1200 Series) as shown in Figure 2. Post-injection chromatogram results were taken and evaluated. After completion of the analysis, the HPLC was purified with mobile phase aid.

HPLC Analysis Conditions:

- ***Column:*** Agilent Zorbax SB-C18, 5 µm pore diameter, 4.6 mm x 250 mm
- ***Mobile Phase:*** 65 parts Ultra-Pure Water, 17.5 parts Acetonitrile and 17.5 parts Tetrahydrafuran
- ***System Flow Rate:*** *1 ml / min*
- ***Wavelength:*** 207 nm
- ***Analyses Duration:*** 5 min

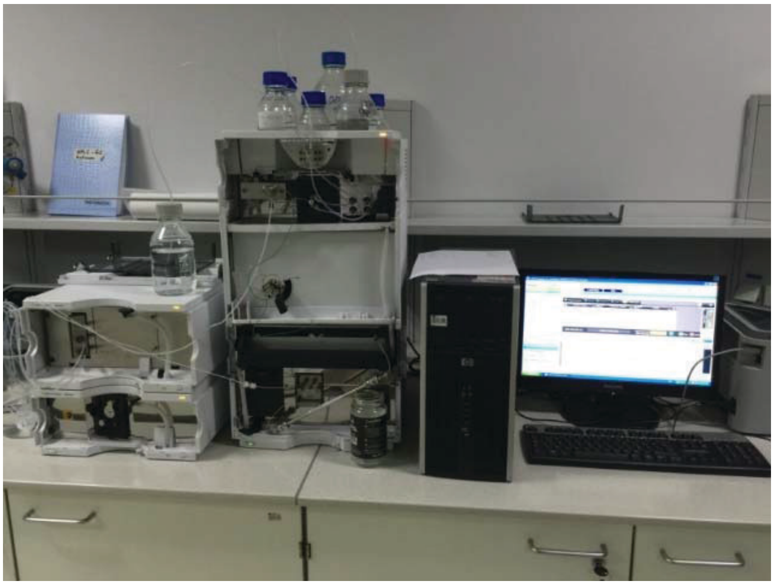

Figure 2. HPLC (Agilent Technologies 1200 Series)

2.2. The Chemical analysis

2.2.1. Titration acidity

The titration acidity was analyzed according to TS 1125 ISO 750 "Fruit and Vegetable Products - Titratable Acid Retention" [30]. 25 mL of pre-treated orange juice was diluted to 250 mL, then 50 mL of diluted orange juice was added and 0.25-0.5 mL of phenolphthalein was added and titrated until a permanent pink color was maintained for 30 seconds with adjusted NaOH solution.

2.2.2. Water soluble matter

The amount of water soluble solids (WSDM) was measured by Abbe refractometer (Reichert). The results were expressed in ° Brix [31].

2.2.3. pH analysis

The pH measurements were determined by pH meter (inoLab WTW pH 720) using orange juice, which was squeezed out and pulverized and heat-treated [26].

2.2.4. The Sensory analysis

In order to determine the changes in the sensory properties of the orange juice samples in the process of eliminating limonin, orange juice samples which had both the juice removed and the juice removed were taken in consideration of "Color-Appearance" and "Bitterness" in the presence of a panelist group of 10 persons. A sorting test was performed according to the sensory evaluation form [32,33].

2.2.5. Statistical analysis

The data obtained from the reduction of citric acid content of orange juice were subjected to analysis of variance with SPSS statistical analysis program (IBM SPSS Statistics 19.0), and the difference data were determined by ANOVA Duncan multiple comparison method. At the end of the statistical evaluation, the data which differ significantly from each other according to the confidence interval of 0.05 was marked with different letters in the respective charts.

2.3. The Calculation of kinetic values

2.3.1. The Calculation of reaction rate (constant k)

In order to calculate the reaction rate constant k, a linear regression analysis was performed according to the curve and the equation of the curve was calculated by placing the limonin changes determined by HPLC on the y-axis and the temperature values on the x-axis. This process was performed for varying the amount of limonin by applying different temperatures, and the reaction rate constant was calculated by determining the gradient by the obtained regression equation [34].

In zero-degree reactions; k= slope (1)

In the first- degree reactions; k=slope*(2.303) (2)

2.3.2. The Calculation of activation energy

The activation energy, which is the effect of the temperature on the reaction rate was calculated by using the Arrhenius equation [35].

$$k = A.e^{(-E_a/R*T)} \quad (3)$$

A= frequency factor constant (the rate of collision and the fraction of collisions with the proper orientation for the reaction to occur)

k= the rate constant
R= the gas constant (1.987 cal/mol. K)
T= Sıcaklık (Kelvin)
E_a= the activation energy (cal/mol or J/mol)

In the reaction, the ln values of k were taken and linear curve was formed by translating the graphs to the y axis, the corresponding temperature values to Kelvin and the reciprocal (1 / T) to the x axis. The regression analysis was performed on the generated curve and the activation energy (Ea) was calculated by multiplying the gradient of the graph by the gas constant [36,37].

$$\text{In } k = - E_a/RT + \text{In}A \quad (4)$$

$$\ln k = -(E_a/R)*(1/T) + \ln A \quad (5)$$

The Temperature Coefficient (Q_{10}) Determination

The Q_{10} value, which indicates the dependence of the reaction on the temperature, was determined by the Eqn. 6 [36].

$$Q_{10} = (k_2/k_1)^{10/(T2-T1)} \quad (6)$$

T_1 & T_2: Reaction temperatures (K)
k_1: The reaction rate constant at T_1 temperature
k_2: The reaction rate constant at T_2 temperature

3. Results and Discussion

The findings related to sensitivity and sensitivity of application of debittered and fresh orange juice in the study of removal of limonin by application of adsorbent in orange juice were included in this section.

3.1. The Calibration curve of the limonin solution

Before analyzing the debittered and fresh orange juices by using HPLC, limonin standards were prepared at different concentrations and the calibration curve generated was shown in Figure 3.

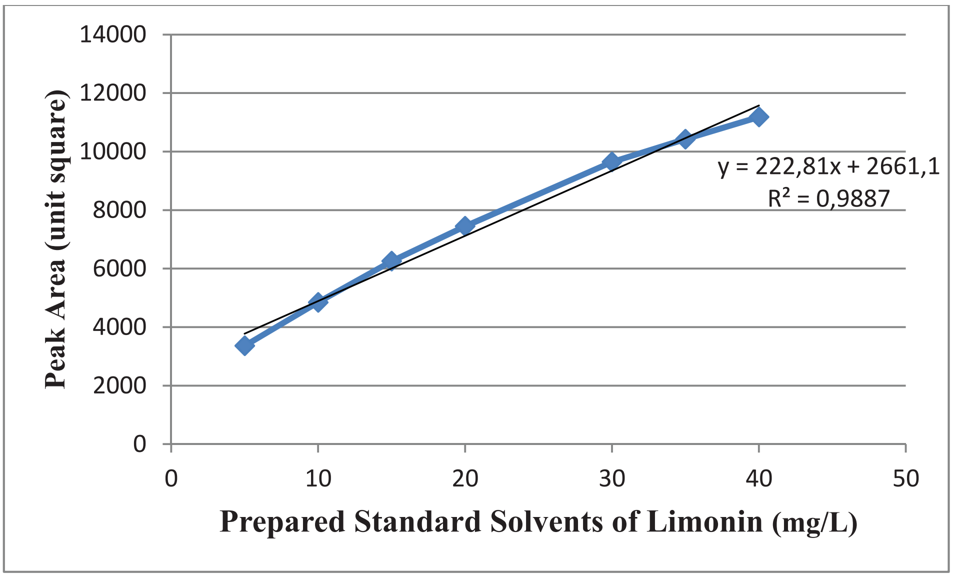

Figure 3. The regression curve and regression equation of prepared limonin solvent

3.2. The Limonin quantity of amberlite applied orange juice

The results of analysis of elimination of limonin sorption by adsorbent of orange juice are given in Tables 1 and 2. The results of the studies performed in different runs (1st and 2nd runs) and at different temperatures (30, 40, 50 and 60ºC) and statistical analyzes by using SPSS program are given in Tables 1 and 2. The amount of limonin in the first run were reduced %90, %89% %80 and %47 at temperature of 30^0C, 40^0C, 50^0C and 60^0C, respectively. It was observed that the treatment of amberlite applied orange juice at 30 ºC significantly differed from the other temperatures by applying different temperatures in the first run to remove limonin bitterness ($p<0.05$) as shown in Table 1. It was determined that the treatments performed at 40ºC, 50ºC, 60ºC, were not caused any significant difference ($p>0.05$). In the second run, 45%, 96%, 84% and 80% reduction at 30ºC, 40ºC, 50ºC and 60ºC, respectively. In the second run, it was concluded that the orange juice applied by amberlite was significantly different from the other temperature applications when applied at 40 ºC ($p<0.05$) as shown in Table 2. It was also determined that the operations performed at 30ºC, 50ºC, 60ºC did not make any significant difference ($p>0.05$).

Table 1. Analysis results of 1st run at different temperatures in removing limonin bitterness with adsorbent applied orange juice

1st Run		
Temp (ºC)	**Amount of limonin in orange juice (ppm)**	**Amount of limonin in Amberlite Applied Orange Juice (ppm)**
30	$3{,}2^{a}$	$1{,}46^{a} \pm 1{,}16$
40	$9{,}8^{c}$	$7{,}98^{b} \pm 0{,}24$
50	$8{,}2^{b}$	$7{,}93^{b} \pm 0{,}41$
60	$8{,}5^{b}$	$8{,}07^{b} \pm 0{,}44$

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p> 0.05) comparing to the ANOVA Duncan test.*

Table 2. The results of the work done in the 2nd run at different temperatures in the process of eliminating the limonin in the orange juice by using the adsorbent

2nd Run		
Temp (ºC)	**Amount of limonin in orange juice (ppm)**	**Amount of limonin in Amberlite Applied Orange Juice (ppm)**
30	11^{a}	$9{,}8^{b} \pm 0{,}84$
40	$11{,}4^{a}$	$5{,}4^{a} \pm 0{,}59$
50	11^{a}	$10^{b} \pm 0{,}84$
60	$12{,}5^{b}$	$10^{b} \pm 0{,}43$

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test.*

In addition, the percent reduction curves of orange juice from 1st and 2nd runs in Table 1 and Table 2 are given in Figure 4 for the second run in Figure 4 and Figure 5 for the 1st run. It was determined that the percentage reduction in the amount of limonin at the different temperatures applied was at most 30°C in the 1st run.

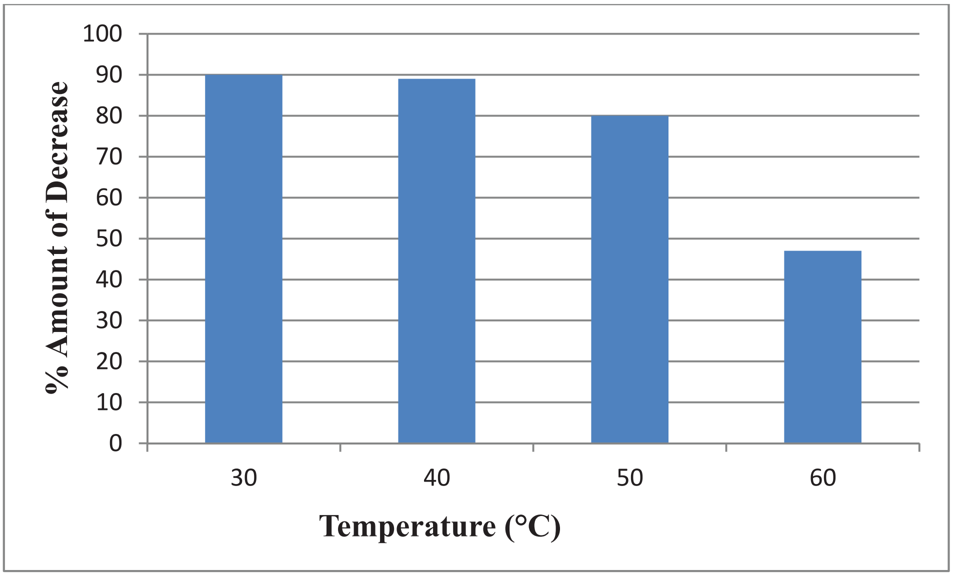

Figure 4. The percentage reduction in the amount of limonin at the different temperatures in the 1st run

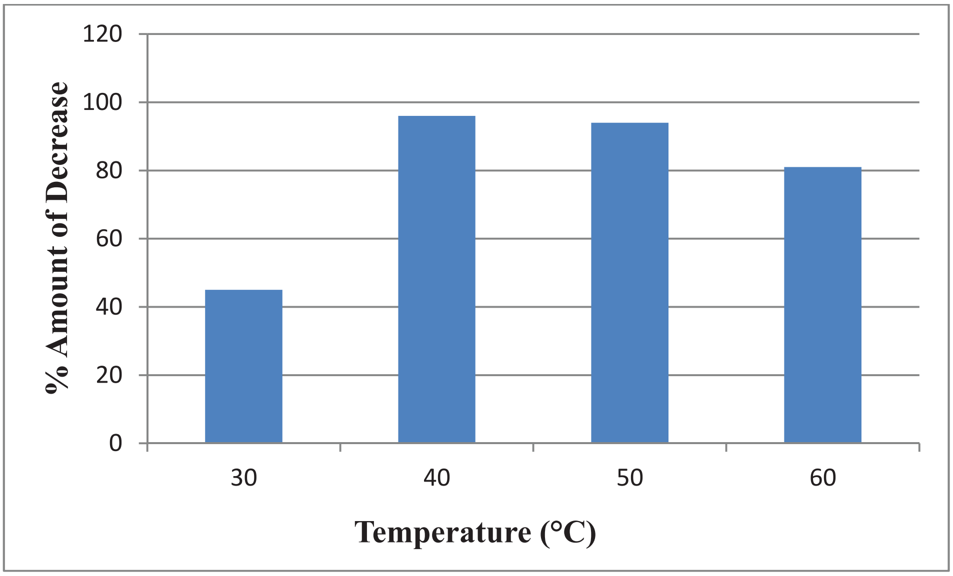

Figure 5. The percentage reduction in the amount of limonin at the different temperatures in the 2nd run

It was observed that the decrease in the amount of limonin was the highest at 40ºC in the experiment of eliminating the bitterness at different temperatures with the experimental adsorbent. It has been reported that Amberlite XAD-4 [38] and Amberlite XAD-16 [39] resins can be used in both citrus juice and low cost juice production. In addition, in the study by Lee and Kim [10], bitterness was tried by using ultrafiltration + polystyrene divinyl benzene in concentrated red grapefruit juice, and the redness of red grapefruit juice decreased by 80-90%. This preferred adsorbent was found to have a linear relationship with adsorption of limonin through its physical properties such as cross-linking ratios, pore diameters and specific surface areas as mentioned by Lee and Kim [10]. In addition, orange juices from Washington (navel) oranges were treated with different adsorbents (Amberlite XAD16 and Dowex Optipore L285) at different temperatures (20ºC, 35ºC and 50ºC) and the amount of limonin were investigated by Kola et al. [39]. Similar results also had been reported by Kola et.al. [39], as they were found that limonin content was reduced by 95-99%

Table 3. Water Soluble Dry Matter analysis results in the 1st run

1st Run		
Temp (ºC)	**Orange Juice (Brix)**	**Amberlite Applied Orange Juice (Brix)**
30	11,5± 0.0^a	11,4± 0.05^a
40	11,5± 0.0^a	11,5± 0.05^a
50	11,5± 0.0^a	11,5± 0.0^a
60	11,5± 0.0^a	11,5± 0.0^a

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test.*

Table 4. Water Soluble Dry Matter analysis results in the 2nd run

2nd Run		
Temp (ºC)	**Orange Juice (Brix)**	**Amberlite Applied Orange Juice (Brix)**
30	12,1± 0.0^a	12± 0.0^a
40	12,1± 0.0^a	12± 0.0^a
50	12,1± 0.0^a	12,1± 0.05^a
60	12,1± 0.0^a	12± 0.0^a

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test.*

In this study, it was determined that the orange juice was reduced by 90% at 30ºC in the 1st run and 96% at 40ºC in the 2nd run by using a different form of the adsorbent (Amberlite XAD-16) used (Amberlite XAD-7HP). In addition, despite the use of different forms of adsorbent, approximate results were obtained. Furthermore, it was determined that Q_{10} value was directly proportional to the activation energy. It was concluded that the run was slower because of higher activation energy values. The results of water soluble dry matter analysis of orange juice treated with adsorbent at different temperatures and at different runs in the study of removing orange juice caused by the use of adsorbent were given in Table 3 and Table 4. As shown in Table 3. and Table 3, it was observed that the WSDM values increased with time and no significant change was observed after the adsorbent application. In both runs it was observed that the temperature did not make any significant difference in the dry matter analysis results (p>0.05).

Table 5. The results of titration assay analysis at different temperatures and in the 1st run to eliminate limonin bitterness with adsorbent in orange juices.

1st Run		
Temp (ºC)	**Orange Juice**	**Amberlite Applied Orange Juice**
30	1,43± 0.0^a	1,40± 0.0^a
40	1,43± 0.0^a	1,40± 0.0^a
50	1,43± 0.0^a	1,39± 0.0^a
60	1,43± 0.0^a	1,40± 0.0^a

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test.*

Table 6. The results of titration assay analysis at different temperatures and in the 2nd run to eliminate limonin bitterness with adsorbent in orange juices

2nd Run		
Temp (ºC)	**Orange Juice**	**Amberlite Applied Orange Juice**
30	1,33± 0.0^a	1,30± 0.0^a
40	1,33± 0.0^a	1,30± 0.0^a
50	1,33± 0.0^a	1,29± 0.0^a
60	1,33± 0.0^a	1,29± 0.0^a

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test*

The titration acidity results of orange juice treated with orange juice at different temperatures for the removal of limonin caused by the adsorbent in orange juice are given in Table 5 and Table 6. Tables 5 and 6 show that the titratable acidity values of the different runs decreased with time in the bittering process at different temperatures by applying adsorbent. It was observed that the acidity values didn’t change because of the increasing of temperature. The acidity values changed with the amberlite application. In the first and second runs, it was observed that there was no significant difference in the titration acidity analysis results of the temperature (p>0.05) as shown in Table 7&8.

Table 7. The results of pH analysis at different temperatures and in the 1st run to eliminate limonin bitterness with adsorbent in orange juices

1st Run		
Temp (ºC)	**Orange Juice**	**Amberlite Applied Orange Juice**
30	3,35± 0.0[a]	3,42± 0.0[a]
40	3,34± 0.0[a]	3,40± 0.0[a]
50	3,34± 0.0[a]	3,40± 0.0[a]
60	3,35± 0.0[a]	3,41± 0.0[a]

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test*

Table 8. The results of pH analysis at different temperatures and in the 2nd run to eliminate limonin bitterness with adsorbent in orange juices

2nd Run		
Temp (ºC)	**Orange Juice**	**Amberlite Applied Orange Juice**
30	3,40± 0.0[a]	3,42± 0.0[a]
40	3,40± 0.0[a]	3,41± 0.0[a]
50	3,40± 0.0[a]	3,41± 0.0[a]
60	3,41± 0.0[a]	3,42± 0.0[a]

** The same letters in the same column indicate that the difference in the amount of limonin in the orange juice was not statistically significant (p>0.05) comparing to the ANOVA Duncan test.*

Table 9. Data for the Arrhenius Graph for different temperatures in the 1st run for orange juice

1st Run				
Temp (ºC)	**(K)**	**k**	**-Ink**	**1/Tx10³**
40	**313**	6,49	1,87	3,19
50	**323**	7,28	1,98	3,09
60	**333**	7,63	2,03	3,00

Finally, the bitterness was removed and the pH was analyzed in the orange juices which had not been relieved, and the results were shown in Table 7 and Table 8. As a result of this study, it was determined that the pH change increases with time and temperature. In both runs, it was observed that the temperature did not make any significant difference in the pH analysis results ($p>0,05$).

3.3. The Evaluation of kinetic values of limonin amount

The concentration values of the amount of limonin in the orange juice were calculated in ppm, and a graph was drawn with k constant found as a result of the operations performed.

After the graph was drawn, the comparison of the regression coefficient calculations and the determination (R^2) values was made as shown in Tables 9 and 10.

As a result of these calculations, the increase in the value of k together with the temperature was observed in Tables 9 and 10. The Arrhenius graphs at the different temperatures in the 1st and 2nd runs for orange juice were shown in Figure 6 and Figure 7.

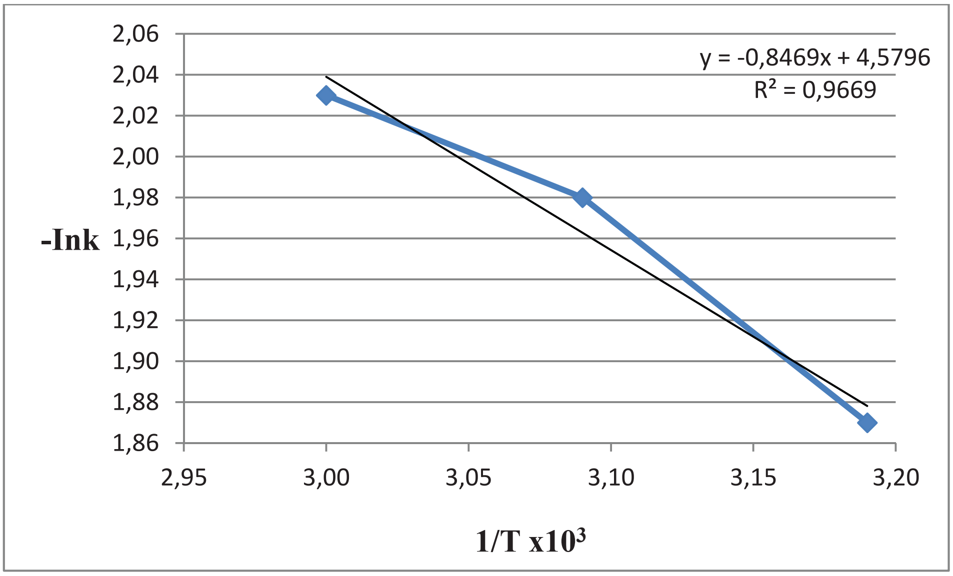

Figure 6. The Arrhenius Graph at the different temperatures in the 1st run for orange juice

Table 10. Data for the Arrhenius graph for different temperatures in the 2nd run for orange juice

2nd Run					
Temp (°C)	**K**	**k**	**-Ink**	**1/Tx10³**
40	**313**	2,55	0,93	3,19
50	**323**	8	2,07	3,09
60	**333**	9,09	2,2	3,00

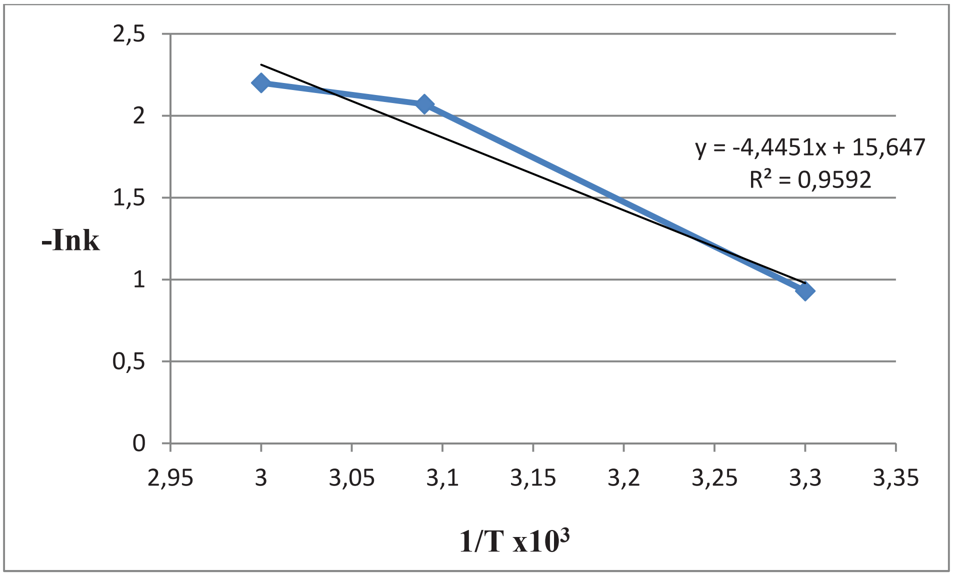

Figure 7. The Arrhenius Graph at the different temperatures in the 1st run for orange juice

3.4. The Q_{10} value results

The effect of the reaction rates on the temperature of 40-60ºC of the orange juices with activation energies was explained while the Q_{10} values were calculated between 40-50ºC and 50-60ºC and the effect of temperature was evaluated and the results are shown in Table 11.

Table 11. Kinetic data on the amounts of limonin in orange juices obtained at different temperatures and times

	E_a		Q_{10}	
	kcal/mol	**kj/mol**	**40-50°C**	**50-60°C**
I. Run	1,68	7,02	0,79	1,05
II. Run	8,83	36,90	3,13	4,13

The changes in the amount of limonin in the orange juices obtained by different temperatures were investigated and the activation energies were found as 1.68 kcal / mol and 8.83 kcal / mol for the first and second runs, respectively. The higher activation energy indicates that the reaction will begin more difficult and progress more slowly. In other words, a slower reaction is observed because the activation energy of the orange juices obtained in the second run is higher than in the first run. Q_{10} values vary between 0.79-1.05 for the first run and 3.13-4.13 for the second run. It was observed that the Q_{10} values caused a 2-fold increase in temperature changes, which was 1 fold. The results indicated that Q_{10} values were directly proportional to the activation energy

4. Conclusion

In this study, the limonin which caused bitter flavor in the orange juices obtained from Washington (navel) oranges was resolved by applying adsorbent at different temperatures. The removal of limonin bitterness was determined as 90% at 30ºC and 96% in 40ºC in this experimental runs. It can be concluded that pH of orange juice and WSDW values were not changed but the acid value was changed related with temperature and time.

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