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Thermal degradation kinetics of rutin and total phenolic compounds in rosehip (*Rosa canina L.*) nectar

Kuşburnu (*Rosa canina L.*) nektarında rutin ve toplam fenolik bileşiklerin ısıl parçalanma kinetiği

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Abstract

In this research it is attempted to determine the loss of rutin and total phenolic compounds in rosehip nectar with the heating periods (0, 5, 10, 15, 20 and 30 min) at temperatures ranging from 70 to 95 °C. Spectrophotometric and liquid chromatographic (HPLC) methods were used to determine the concentrations of total phenolic compounds and rutin, respectively. Thermal degradations of TPC and rutin in rosehip nectar were fitted to a first-order reaction kinetic model. Arrhenius relationship was used in order to describe the temperature dependence of reaction. Activation energies for rutin and TPC between 70 to 95 °C were found to be 46.90 and 35.72 J/mol, respectively.

Keywords: Degradation kinetics, HPLC, Phenolic, Rosehip nectar, Rutin, Water- soluble vitamin

Öz

Bu çalışmanın amacı, kuşburnu nektarının 70 ile 95 °C sıcaklık aralığında periyodik olarak farklı süreyle (0, 5, 10, 15, 20 ve 30 dk.) ısıl işleme tabi tutulmasına bağlı olarak rutin ve toplam fenolik bileşik içeriğinde meydana gelen değişimin belirlenmesidir. Kuşburnu nektarının rutin içeriği sıvı kromatografisi (HPLC) ve toplam fenolik madde miktarı spektrofotometrik yöntemle analiz edilmiştir. Isıl işlem sırasında, kuşburnu nektarında ısıl işlem uygulamasına bağlı olarak rutin ve TFB içeriğinin bozunması birinci dereceden kinetik modele uymuştur. Reaksiyonun sıcaklık bağımlılığı Arrhenius ilişkisi ile tanımlanmıştır. Rutin ve TFB içeriğinin 70 ile 95 °C sıcaklık aralığındaki aktivasyon enerjileri sırasıyla 46.90 ve 35.72 J/mol olarak bulunmuştur.

Anahtar kelimeler: Parçalanma kinetiği, HPLC, Fenolik, Kuşburnu nektarı, Rutin, Suda çözünen vitamin

1 Introduction

The main native land of Rosehip (*Rosa canina L*), belongs to the plant family of Rosaceae, is West Asia-North Europe [1],[2] and Middle East-North-East Anatolia of Turkiye [3].

Rosehip is a good source of some biologically active compounds. The main importance of rosehip is its high composition of vitamin C and rutin (vitamin P) and high content of pectins, fatty acids, sugars, organic acids, phenolic components, lycopene, carotene with activity of vitamin A, group B vitamins and vitamin K, tannins, carotenoids, minerals (particularly K and P) macro- and microelements [4]-[8].

Rosehip is the fruit with the highest level of vitamin C, as well as an important source of rutin (vitamin P). The other important food sources of rutin is buckwheat, onion, lemon, apple, orange, and grapefruit [9] and tea [10]. Rutin, a compound of the flavonoid class as flavanoid glycoside, consist of quercetin and the disaccharide rutinose [11]-[12] and has antioxidant [13], antiinflammatory [14], neuroprotective [12],[15], antihyperglycaemic [16] and antimicrobial [17] activities.

Phenolic compounds are separated into two groups as phenolic acids and flavonoids. Flavonoids are polyphenolic antioxidants found in natural herbal teas, fruits and vegetables [18]. Some of the phenolic compounds are effective in the formation of the flavor components of fruits and vegetables, particularly in the formation of two important flavors such as bitterness and sourness in the mouth. Some of them provide the colors of fruits and vegetables in yellow, yellow-brown, red-blue tones [19],[20]. Phenolics have antioxidant, antimutagenic and anticarcinogenic effects and are capable of altering gene expression [21],[22]. Roseships are very rich in phenolics [23]. The total phenolic content (TPC) of *Rosa canina* has been found to be 96 mg gallic acid equivalent/g dry weight [22].

Several analytical techniques such as spectrophotometric [24] and liquid chromatographic [25]-[29] have been tested for rutin analysis. HPLC is the preferential method for rutin separation in terms of rapid, accurate and sensitive quantitative determination.

While maintaining quality factors in maximum level in safe food production, kinetic models for thermal treatments are necessary in food production designs [30]. Thus, technological control and prediction of quality in foods can be achieved by kinetic modeling of food changes [31]. As far as we know, no report was published about the HPLC determination of rutin content of rosehip pulp or nectar and thermal degradation of rutin and TPC of rosehip nectar. In this study it was aimed:

a) To determine the rutin and TPC changes during thermal treatments (70, 80, 90 and 95 °C),

b) To determine the degradation kinetics of rutin and TPC in rosehip nectar with different thermal processing (0, 5, 10, 15, 20, 25 and 30 minutes) at temperatures between 70 and 95 °C,

c) To define degradation reactions with the determination of kinetic parameters (reaction rate constant, order of reaction, activation energy, Q_{10}, half-life).

2 Material and methods

2.1 Fruits

The dried rosehip fruits (*Rosa canina L*) were provided from a well-Established local factory (Gümüssu Food Co., Gümüshane) in eastern Black Sea Region of Turkey. Nearly 250 kilograms of dried fruits were transferred in sacks to the laboratory of Pamukkale University, Denizli, Turkey and processed for rosehip nectar.

2.2 Production of rosehip nectar

The rosehip fruit, which is washed in under tap water in a clean container, is ground by a fruit grinder. During the milling of the fruit, about 1-1.5 times the water weight of the fruit is added [32]. After mashing at 70 °C for 30 minutes, rosehip pulp was obtained by passing through screens of 1.6 and 0.4 mm pore size. According to Turkish Food Codex, fruit nectar is defined as an unfermented product prepared with addition of water, sugar or honey into fruit pulp or puree. The pulp was processed to nectar by sugar, citric acid and water supplement up to 12-13 brix with minimum 40% puree and maximum 10% sugar. So, the acidity and water-soluble matter of the pulp were adjusted to 5.5 g/l and 16% to obtain rosehip nectar, respectively. After cooling in an ice-water bath at 20-25 °C, the nectar was transferred to pyrex tubes (75x10 mm ID) for the treatment of thermal degradation and stored at 4 °C until thermal processing.

2.3 Thermal treatment

The thermal treatments of rutin and TPC were studied at 70, 80, 90 and 95 °C. Rosehip nectar samples of 25 mL were heated in pyrex tubes (75 x 10 mm ID) placed in a thermostatic water bath (Model 3047, Kottermann, Hänigsen/Germany). Time application was started after the temperatures of the samples measured by thermocouple reached the desired temperature. It took less than 8 minutes to reach the desired temperature in all heating treatments. The caps of the tubes were tightly closed and placed in a thermostatic water bath. After removal of nectar samples at regular periods they were quickly cooled under running water. Rutin and TPC were determined using three test tubes removed from the thermostatic water bath every 5 minute. All results were performed in triplicate and the reaction rate constants of each temperature were calculated in triplicate.

2.4 Selection of temperatures and heating periods

Depending on the heating time, about 70 °C is the lowest value used in industrial-scale production of rosehip nectar. On the other hand, in the conventional production method used in Turkey, the bottle nectar is left in boiling water for about 20-30 minutes in open type boilers. Thus, the temperature of rosehip nectar in the bottle reaches about 95 °C.

2.5 Analysis of rutin

2.5.1 Equipment

A liquid chromatography (Shimadzu corporation, Kyoto, Japan) system consisting of a UV-VIS DAD detector, (Model SPD-M10 AVP, Shimadzu), a column oven (Model CTO-10ASVP, Shimadzu), a quadruple liquid chromatography pump (Model LC-10AT-VP, Shimadzu), a degasser (Model DGU 14A, Shimadzu) and a Shimadzu Software Program was used for the analysis. Additionally, a reversed-phase Discovery C_{18} column (15 cm x 4.6 mm ID, 5μm particle size) (Cat. No:

504955) from SUPELCO (Bellefonte, PA, USA) was used in the HPLC system.

2.6 Reagents

HPLC grade methanol and extra pure potassium dihydrogen phosphate were supplied from Merck (Darmstadt, Germany). Doubly distilled and deionized water was used in the experiments. Analytical-reagent grade standard of rutin was obtained from Sigma Chemical Company (Deisenhofen-Germany). Five different concentrations of rutin standard prepared in mobile phase were used for the preparation of calibration curve. All solutions were stored in dark glass flasks in refrigeration against the negative effects of light. Thus, five point calibration curve, covering the range of 5-250 ppm with the correlation coefficients of 0.999 based on the concentration (mg/L) versus peak area (mAU), was prepared by using stock-standard solutions of rutin. Triplicate injections with each standard solution were made for the preparation of calibration curve.

2.7 Sample preparation (Solid phase extraction=SPE)

Many components of rosehip cause chromatographic interferences with rutin in HPLC. Therefore, a SPE with Sep-Pak C18 (500 mg) cartridges that let the separation of rutin and remove most of the interfering components were used in sample preparation. A 25 g of deionized water were added into 5 g rosehip nectar (Dilution factor, F=6). Following the homogenization of the mixture using a homogenizer at medium speed for 1 min, homogenized mixture was centrifuged for 10 min at $14x10^3$ rpm (Model 2-16, Sigma Bioblock Scientific). The extraction of rutin was carried out with some modification of SPE method [33]. A 10 ml methanol and 10 ml water adjusted at pH 4.2 was used in flushing for the activation of stationary phase. Following the activation of stationary phase, both homogenized and centrifuged rosehip nectar (10 mL) was loaded. The pH value of the acidified water was prepared by the addition of 0.005 M HCl solution drop by drop until a predetermined pH value was reached. The sample was eluted with water (5 mL, pH 4.2) then 10 ml methanole at a flow rate of 1ml/min. Following the elution, eluents were collected in a bottle and evaporated to dryness. After the dissolving of the residue in mobile phase, aliquot of 20 μl, filtered with 0.45 μm pore size filters (Schleicher-Schuell, Darmstadt-Germany), was injected into the HPLC column for the quantitative determination of rutin.

2.8 Methods

A photodiode array detector set at 204 nm for the determination of rutin was used to achieve the column eluate monitoring. Following the degassing of mobile phase by sonication, filtered through a 0.45 μm filter prior to use. The elution solvents, used in the HPLC analysis, were 0.1 mol/L potassium dihydrogen phosphate (pH: 7) and methanol (90:10) with the flow rate of 0.7 ml/min. The column was operated at 25 °C. Thus, chromatographic data on the peaks were integrated up to 12 min. Integrated area for sample and the corresponding standard was used for the calculation of the rutin concentration.

2.9 Recovery of rutin

Rosehip nectar samples containing known amount of rutin was spiked with the two addition levels of standard rutin for the determination of recovery. For this purpose, six injections were carried out for each addition level.

2.10 Calculation of kinetic parameters

A general equation for thermal degradation kinetics can be written as given below: [34].

$$-\frac{d[C]}{dt} = k[C]^m \tag{1}$$

Where,

C : quantitative amount (mg/L) of the constituent,
k : reaction rate constant,
m : order of the reaction.

After the integration of Eq. (1), the equation for first order kinetics can be written as:

$$\ln\left(\frac{[C]_t}{[C]_0}\right) = -kt \tag{2}$$

Where, $[C]0$ and $[C]t$ are the initial and residual vitamin, respectively. k is the reaction rate constant (1/min) and t represents the time (min.).

Temperature dependence of rutin and TPC was described by the Arrhenius equation 3, given below:

$$k = k_0 \times e^{-E_a/RT} \tag{3}$$

Where, k is the rate constant (milligrams rutin or TPC per min), k_0 the frequency factor (per min), E_a the activation energy (kilojoules per mole), T the absolute temperature (K), and R is the universal gas constant (8.314 x 10^{-3} kJ/mol K):

Quotient indicator (Q_{10}) is another technique for the expression of the dependence of reaction rate on temperature and calculated with the equation 4:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2-T_1}} \tag{4}$$

Where, T is temperature in C, k_1 and k_2 are the rate constants of rutin and/or TPC degradation at temperatures T1 and T2, respectively.

The time required degrading 50% of original concentrations of rutin and TPC was calculated using the equation 5 given below:

$$t_{1/2} = 0.693/k \tag{5}$$

Where, k is the reaction rate constant (per min).

2.11 Analysis of TPC

TPC of rosehip nectar was determined by using the Folin Ciocalteu spectrophotometric method. For this purpose, 40, 80, 120, 160 and 200 ppm concentrations of gallic acid solutions were prepared. With 75 ml of distilled water, 1 ml of sample was mixed in a volumetric flask (100 ml) followed by the addition of 5 ml Folin-Ciocalteu and let to stand for 3 min. Then, saturated sodium carbonate solution (10 mL, 20%) was added and shaken well again. The volumetric flask was then filled with distilled water up to 100 ml. A UV/VIS spectrophotometer (T80, PG Instruments, UK) was used for reading the absorbance of the resultant solution at 720 nm after 1 h standing in a dark place. Quantification was based on the standard curve of gallic acid (0-200 mg/L) which was dissolved in deionized water and expressed as mg gallic acid equivalent (GAE) per liter.

2.12 Further determinations

AOAC [35] method was used for the analysis of total solid (%), water soluble solid (Bx), pH and total acidity (dry tartaric acid). The amounts of total sugars in the rosehip nectar were determined according to the Lane-Eynon method [36].

2.13 Statistical analysis

SAS® software [37] was used for the statistical analysis of all data. Data means were compared using least significant difference test, when analysis of variance (ANOVA) showed a significant effect (P<0.05).

3 Results and discussion

In the world literature, the number of studies on rutin values and rutin changes of foodstuffs is limited and studies are mostly in the field of medicine. However, as far as we know, there have been no degradation kinetic studies on the rutin and TPC and HPLC determination of rutin in rosehip nectar. This work is important to complete lack of the world literature.

As known, each component has a wavelength that gives maximum absorbance such as rutin. So, the detection wavelength was set at max absorption wavelength of rutin for higher sensitivity. As shown in Figure 1, rutin is separated well and a good separation achieved in 12 min. Compound identification was achieved by the comparison of its retention time value and UV spectra with the standard reference compound of data bank. Two unknown peaks were also detected in the chromatogram. However, no interference between the rutin and unknown peaks were observed.
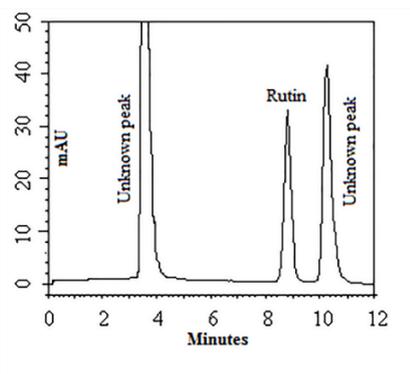

Figure 1: Separation of rutin by isocratic elution with potassium dihydrogen phosphate (pH: 7) and methanol (90:10).

3.1 Analytical characteristic of the HPLC method

3.1.1 Linearity, Detection limit, Recovery and precision

Linearity of standard curve, detection limit, precision and recovery of method for determination of rutin in rosehip nectar is shown in Table 1. An R value of 0.999 was obtained for rutin. Coefficient of determination (r^2) was determined as 99.82%. The detection limit for rutin, based on S/N (signal/noise) of 3 [38], was 0.5 ppm. The method reliability was confirmed by two recovery experiments. Standard addition procedure was used for the recovery test. Thus, 30 and 50 mg/L concentrations of rutin standards were added to the samples. In each addition level, six determinations were realized. The average percentage recovery of rutin in rosehip nectar was determined as 102.3±0.54%. The method precision was evaluated using the same reagents and apparatus under the same experimental conditions with six determinations of the same rosehip nectar sample. In addition, intra- and inter-day tests were applied for the calculation of precision and the results were expressed as relative standard deviation (RSD, %). The evidence of good precision for HPLC is low RSD value that determined (2.20%) in our study. In addition, the low RSD value also shows non-variability of the data.

3.2 Characteristics of rosehip nectar samples used

The characteristics of the rosehip nectar used in the study are as follows: 13.9±0.04% total solid, 12.75±0.35 Bx soluble solids, 3.85±0.07 pH value, 0.35±0.01% total acidity and 12.29±0.02% total sugar, 268.4±4.0 mg/L rutin and 2294±0.1 mg GAE/L TPC. Soluble solids, pH and TPC of rosehip nectar (glass-bottled) were reported as 16.2 Bx, 3.50 and 1967 mg catechin per liter, respectively (Duru et al. 2012).

3.3 Thermal degradation of TPC and rutin in rosehip nectar

Thermal stability of TPC and rutin in rosehip nectar were studied at 70, 80, 90 and 95 °C. As seen from Figure 2 and 3, the degradations of TPC and rutin are fitted to first-order kinetic model. As the heating temperature and time increased, the degradation of TPC and rutin increased. A linear relation, which shows the plot for TPC and rutin in rosehip nectar, correlates to first-order degradation kinetics with the correlation coefficient above 0.98 for all cases. It is pointed out that statistical differences between zero or first-order reactions may be insignificant [34] On the other hand, there are no published data on the thermal degradation of TPC and rutin in rosehip nectar. So, we didn't compare our results with the literature.
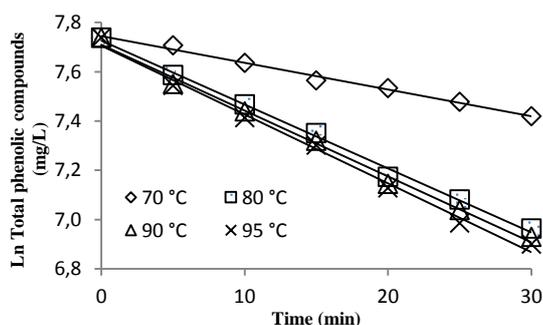

Figure 2: First order kinetic of TPC thermal degradation in rosehip nectar at four different temperatures.
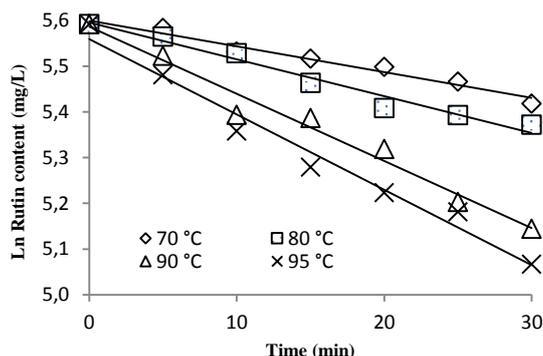

Figure 3: First order kinetic of rutin thermal degradation in rosehip nectar at four different temperatures.

Table 2 shows the degradation rate constants (k) and half-lives ($t_{1/2}$) for TPC and rutin in rosehip nectar. The linear inclination of the plot of ln(C/C0) against t was used in order to estimate the degradation rate constant. The rate constants of TPC and rutin increased with the increment of temperature. This result suggests that degradations of TPC and rutin are temperature-dependent. TPC indicated the minimum degradation rate constant followed by rutin, suggesting that rutin is less inclined to degradation than TPC. The rate constant of rutin increased slightly with the increment of temperature from 70 to 80 °C and

90 to 95 °C, but increased significantly from 80 to 90 °C. The results imply that rutin was very unstable at 95 °C as approved by higher k values resulting a fast degradation. In contrast, the rate constant of TPC increased significantly from 70 to 80 °C and increased slightly with the increament of temperature from 80 to 90 °C and 90 to 95 °C., The k values of rutin and TPC varied between 5.6×10^{-3}-16.5×10^{-3} and 10.8×10^{-3}-28×10^{-3} at temperatures between 70 and 95 °C, respectively (Table 1). Consequently, degradation of TPC and rutin increased with the increment of heating temperature and time.

As shown in Table 1, with the increment of temperature the half-life values ($t_{1/2}$) for rutin and TPC decreased. According to the $t_{1/2}$ *values,* rutin and TPC degrade faster at higher temperatures. Rutin showed the highest $t_{1/2}$ values when compared with the TPC at the same temperatures. This result indicates the higher thermal stability of rutin at the same temperatures in rosehip nectar when compared with the TPC.

If the logarithm of the rate constants vs reciprocal of absolute temperatures process to a linear coordinate system, the slope of the linear curve gives the value of Ea/R (Figure 4). Activation energies for rutin and TPC degradation in rosehip nectar were different at temperature between 70-95 °C. Rutin showed the highest activation energy, followed by TPC. These results suggest that rutin is more stable to thermal degradation in rosehip nectar compared to TPC. The high activation energy of a reaction indicates that this reaction is very sensitive to temperature change. Therefore, rutin and TPC of rosehip nectar are more susceptible to thermal degradation at 95 °C than 70 °C. The activation energies were calculated as 35.72 and 46.90 J/mol for the degradations of TPC and rutin, respectively.
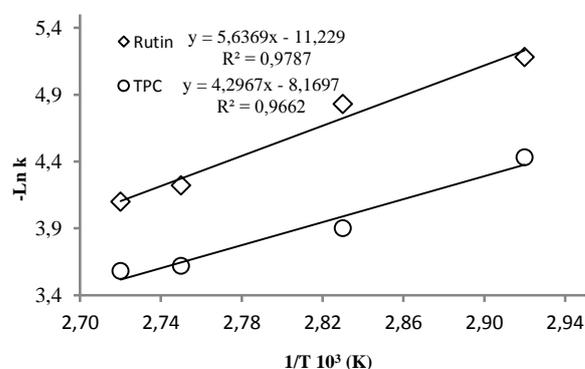

Figure 4: Arrhenius plots for degradation of TPC and rutin in rosehip nectar during heating.

For that reason, the calculated Q_{10} values of rutin and TPC are compared to the literature reported Q_{10} values of other sources. Table 2 shows the Q_{10} values of rutin and TPC in rosehip nectar between the temperatures of 70 and 95 °C. The Q_{10} values, for increases of each 10 °C, were different in rosehip nectar at temperatures ranging from 70 to 95 °C. The highest Q_{10} values for rutin and TPC are obtained within the ranges of 80 to 90 °C and 70 to 80 °C, respectively. This result point out that the thermal degradation of rutin and TPC was highyl affected with the temperatures in the ranges of 80 to 90 °C and 70 to 80 °C, respectively. In the range of 90 to 95 °C, the Q_{10} values of rutin and TPC are the same, indicating that within this range the degradation kinetics are very few affected by the temperature change.

Table 1: Linearity of standard curve and detection limit, recovery and precision of method for determination of rutin in rosehip nectar.

Vitamin	Linear range (mg/L)	R	r^2	Detection limit (mg/L)	Initial content (mg/L)	Content after addition (mg/L)[a]	Recovery (%) Mean S.D[b]	Precision R.S.D. (%)
Rutin	5.0-250.0	0.999	99.82	0.5	248.6 ± 0.9	299.8±0.13	102.3±0.54	2.20

[a]: 50 mg for rutin, [b]: Mean± standard deviation.

Table 2: Effect of temperature on the activation energy (Ea), reaction rate constant (k), Q10 and half-life (t1/2) values of rutin and and TPC.

Vitamin	T (°C)	k 10^3 (1/min)	$t_{1/2}$ (min)	Q10 70-80 °C	80-90 °C	90-95 °C	Ea (J/mol)
Rutin	70	5.6	123.75				
	80	8.0	86.63				
	90	14.7	47.14	1.43	1.84	1.26	46.90
	95	16.5	42.00				
Total Phenolic Compounds	70	10.8	64.16				
	80	25.9	26.76				
				2.4	1.03	1.10	35.72
	90	26.7	25.96				
	95	28.0	24.75				

4 Conclusion

Thermal degradation of rutin and TPC of rosehip nectar heated at various temperatures were evaluated first time in the world. Degradation of rutin and TPC followed firs-order kinetics. As the temperature and heating time increased, degradation rate of examined compounds increased. The best temperature for the less reduction of rutin and TPC of rosehip nectar for long term heating of rosehip nectar was 70 °C. The rosehip nectar producing industry may benefit from the results of this study when controlling the amount of rutin and TPC in production process. The consequences of this research will assist the rosehip nectar industry in minimum loss of rutin and TPC. These parameters are important quality criteria for rosehip nectar. Therefore, knowing the degradation kinetics of rutin and TPC is important. In addition, there is no study on the thermal degradation of rutin and TPC of rosehip nectar. Therefore, this study will shed light on future studies

5 Acknowledgement

6 References

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