Thermal degradation kinetics of ascorbic acid, thiamine and riboflavin in rosehip (Rosa canina L) nectar
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1 Introduction

The Rosehip (Rosa canina L) belongs to the plant family of Rosaceae, and its native area is West Asia and North Europe (Yamankaradeniz, 1983; Artık & Ekşi, 1988) and grows especially well in the middle and North-East Anatolia of Turkey (Davis, 1972).

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 and group B vitamins, tannins, carotenoids, minerals (particularly K and P) macro- and microelements (Tuer & Russel, 1989; Demir & Özcan, 2001; Kadakal et al., 2002; Roman et al., 2013; Abacı et al., 2016). In addition to vitamin C, rosehip contains thiamine, riboflavin, E and K vitamins (Stundzhyä & Shnaidman, 1972; Yamankaradeniz, 1983; Tuer & Russel, 1989). As it is known, rosehip fruit has been used as a medicine due to its high vitamin C content since prehistoric times (Ropciuc et al., 2011).

Rosehip fruits have significance because of its nutritional, physiological and technological properties. Turkey has a rich potential of indigenous and traditional food production techniques. Evaluation form of rosehip consists of application of our main traditional food production methods. This product has exceptional properties in view of its content of carbohydrates, minerals and vitamins. In addition, rosehip plant supplies nutritive elements for human consumption and is believed to have several pharmaceutical properties and is believed to be a protective agent for several diseases (Kadakal et al., 2002).

Vitamins, a broad group of organic compounds for food, are essential for the self-maintenance, functioning and normal growth of the human and animal bodies. Vitamins are separated into two groups, water-soluble and fat-soluble. Processing and storage of food through chemical reactions cause loss of vitamins. Because of the relative unstability and their critical role in nutrition, qualitative and quantitative analysis of vitamins are important issues for food manufacturers (Ottoway, 1993; Moreno & Salvado, 2000). Because of high selectivity thanks to solid phase extraction (removing interfering components), HPLC is the preferred method for the separation of vitamins (Cho et al., 2000; Kadakal et al., 2007).

While maintaining highest level of quality for safe food production, kinetic models of thermal treatment are essential in new food processes designs (Avila & Silva, 1999). Therefore, technological control and prediction of quality in foods can be achieved by kinetic modeling of food changes (Van Boekel, 2008). To the best of the authors’ knowledge, due to lack of study on the thermal degradation of L-ascorbic acid, thiamine and riboflavin in rosehip nectar, this manuscript will be the first reported study to enable future analysis.

Abstract

In this paper, the loss of L-ascorbic acid, thiamine and riboflavin in rosehip nectar with the heating periods (0, 5, 10, 15, 20 and 30 min) at temperatures ranging from 70 to 95 °C is analyzed and experimental results are presented. Firstly, dried rosehip fruits were processed to rosehip nectar and then thermal treatment is performed. Liquid chromatographic (HPLC) method was used for the analysis of the contents of L-ascorbic acid, thiamine and riboflavin and examined compounds are thoroughly separated within 25 min. During thermal processing, degradation of L-ascorbic acid, thiamine and riboflavin in rosehip nectar were fitted to a first-order reaction kinetic model. Arrhenius relationship was used for the description of temperature dependence of reaction. Activation energies for L-ascorbic acid, thiamine and riboflavin between 70 to 95 °C were found to be 55.30, 36.38 and 37.15 kJ/mol, respectively. To the best of the author’s knowledge, due to lack of study on the thermal degradation of L-ascorbic acid, thiamine and riboflavin in rosehip nectar, this manuscript will be the first reported study to enable future analysis.

Keywords: ascorbic acid; degradation kinetics; HPLC; rosehip nectar; water-soluble vitamin.

Practical Application: The thermal degradation of L-ascorbic acid, thiamine and riboflavin had a first-order kinetic model and had a strong temperature dependent. Based on the results obtained, it is determined that 70 °C is the best temperature for minimizing reduction of water-soluble vitamins. On the other hand, while studies on the thermal stability of thiamine and riboflavin in fruit nectars are limited, there is no prior publication for the rosehip nectar. To the best of the authors’ knowledge, this will be the first reported study to shed light on future studies and relevant information about rosehip nectar for consumers and food processors.
of our knowledge, there is no published research study about the HPLC determination of L-ascorbic acid (AA), thiamine (TH) and riboflavin (RB) and thermal degradation of AA, TH and RB of rosehip nectar (RHN). The objectives of this study are: (a) to determine the AA, TH and RB changes during thermal treatments (70, 80, 90 and 95 °C) (b) to determine the kinetics of AA, TH and RB degradation for rosehip nectar with different thermal processing (0, 5, 10, 15, 20, 25 and 30 minutes) over the temperature range of 70 to 95 °C (c) to define the degradation reactions with the determination of kinetic parameters, such as order of reaction, reaction rate constant, activation energy, $Q_{ch}$ and half-life.

2 Material and methods

2.1 Material

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

2.2 Production of RHN

The rosehip fruit is first washed in water in a clean container, then ground by a fruit grinder. During the milling of the fruit, about 1-1.5 times the water weight of the fruit is added (Nas & Gökalp, 1993). 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. After cooling in an ice-water bath at 20-25 °C, the nectar was transferred to pyrex tubes (75 x 10 mm ID) for the treatment of thermal degradation and stored at 4 °C until thermal processing.

2.3 Thermal treatment

The thermal treatments of AA, TH and RB were studied at 70, 80, 90 and 95 °C. RHN samples of 25 mL were heated in pyrex tubes (a three-necked round bottom flask, 75 x 10 mm ID) placed in a thermostatic water bath (Model 3047, Kottermann, Hänigsen/Germany). The application time is started after 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 to prevent evaporation and placed in a thermostatic water bath. After removal of nectar samples from the water bath at regular time intervals they were rapidly cooled in an ice water bath. AA, TH and RB were determined using three test tubes removed from the thermostatic water bath every 5 minute. All experiments were run in triplicate and the reaction rate constants of each temperature were calculated in triplicate also.

2.4 Selection of temperatures and heating periods

70 °C is the lowest value used in the production of industrial scale rosehip nectar depending on the heating time. However, in traditional production method, bottled rosehip nectars are left to boiling water in an open type boilers for approximately 20-30 minutes. Thus, the temperature of rosehip nectar in the bottle reaches about 95 °C.

2.5 Analysis of water-soluble vitamins

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. A syringe (Hamilton Co., Reno, NV, USA) was used for the injection of the sample (20 µL) into the HPLC. Additionally, a reversed-phase discovery C$_{18}$ column (15 cmx4.6 mm ID, 5µm particle size) (Cat. No: 504955) from SUPELCO (Bellefonte, PA, USA) was used in the HPLC system.

Reagents

HPLC grade methanol and extra pure potassium dihydrogen phosphate were supplied from Merck (Darmstadt, Germany). Doubly distilled and deionised water was used in the experiments. Analytical-reagent grade standards of AA, TH and RB were obtained from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen-Germany) and stock-standard solutions of AA, TH and RB were prepared in mobile phase. Calibration curve was prepared with five different concentrations of each standard. Solutions used in the study were first sonicated and stored in dark glass flasks, in order to protect them from light, and then kept under refrigeration. Thus, five point calibration curves with the correlation coefficients of 0.999 based on the concentration (g/ml) versus peak area (mAU) were prepared for AA, TH and RB.

Sample preparation (solid phase extraction=SPE)

Many components of rosehip cause chromatographic interference with AA, TH and RB. Therefore, a SPE with Sep-Pak C18 (500 mg) cartridges that let the separations of AA, TH and RB and suspend 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 14 x 10$^3$ rpm (Model 2-16, Sigma Bioblock Scientific). The extraction of AA, TH and RB was carried out with some modification of SPE method of Cho et al. (2000). Briefly, 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) followed by methanol (10 mL) at a flow rate of 1 mL/min. Following the elution, eluents were collected in a bottle and evaporated to dryness. The residue was dissolved in mobile phase and aliquot of 20 µL was injected into the HPLC column for the quantitative determinations of AA, TH and RB. In addition, FP 30/45 CA-S filters (Schleicher & Schuell, Darmstadt-Germany) with 0.45 µm (7 bar max) pore size were used for the filtration of samples prior to HPLC analysis.

2.6 Methods

The detection of column eluate was achieved with a photodiode array detector at 265 nm for AA, 234 nm for TH and 266 nm for RB, respectively. Following the degassing of mobile phase by sonication the mobile phase was filtered through a 0.45 µm membrane prior to use. The elution solvents, 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 sample injection loop volume of the system was 20 µL and the column was operated at 25°C (room temperature). Thus, chromatographic data on the peaks were integrated up to 25 min. Identification of peak was realized by comparing its retention time value and UV spectra with the standard reference compound stored in a data bank. Integrated areas of the sample and the corresponding standards were used for the calculation of the AA, TH and RB concentrations.

2.7 Recovery of water-soluble vitamins

RHN samples containing known amounts of AA, TH and RB were spiked with the two additional levels of standard AA, TH and RB to determine the recovery. For this purpose, six determinations were carried out for each addition level.

2.8 Calculation of kinetic parameters

A general reaction rate expression for the degradation kinetics can be written as follows (Labuza & Riboh, 1982; Kadakal & Artik, 2008):

$$-d[C] / dt = k[C]^m$$  

(1)

Where [C] is the quantitative value of the component under consideration, k is the reaction rate constant and m is the order of the reaction. Degradations of AA, TH and RB in RHN were determined to fit first-order kinetics. According to this result, the equation for first order kinetics after integration of Equation 1 can be written as:

$$\ln(C_0 / C_f) = -kt$$  

(2)

Where $C_0$ and $C_f$ are the initial and residual AA, TH and RB, respectively, k is the reaction rate constant (per min) and t represents the time (min).

Temperature dependence of AA, TH and RB were described by the Arrhenius Equation 3, given below:

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

(3)

Where k is the rate constant (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 ($8.314 \times 10^{-3}$ kJ/mol K).

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

$$Q_{10} = \left(\frac{k_{10}}{k_T}\right)^{10 / T}$$  

(4)

Where T is temperature in °C, $k_1$ and $k_2$ are the rate constants of rutin and/or TPC degradation at temperatures $T_1$ and $T_2$, respectively.

The time required degrading 50% of original concentrations of AA, TH and RB was calculated using the Equation 5, given below:

$$t_{1/2} = 0.693 / k$$  

(5)

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

2.9 Further determinations

Method of Association of Official Analytical Chemists (1990) was used for the analysis of total solid (%), water soluble solid (Bx), pH and total acidity (dry citric acid). The amount of total sugar in the rosehip nectar was determined according to the Lane-Eynon method (Cemeroğlu, 1992).

2.10 Statistical analysis

"Statistical Analysis Systems" of SAS Institute Inc (1985) 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 TH and RB values and their changes of foodstuffs are limited and published studies are mostly on the AA content of rosehip. However, to the best of our knowledge, there have been no studies on the degradation kinetics and HPLC determination of AA, TH and RB in RHN, the proposed work is a critical contribution to the literature.

As known, each component has a wavelength that gives maximum absorbance such as AA, TH and RB. Therefore, the detection wavelengths were set at max absorption wavelengths of AA, TH and RB for higher sensitivity. As shown in Figure 1, AA, TH and RB are separated well and a good separation achieved in 25 min. Compound identification was achieved by the comparison of its retention time value and UV spectra with the standard reference compound of data bank. Four unknown peaks were also detected in the chromatogram. However, no interference between the AA, TH, RB and the unknown peaks were observed.
3.1 Analytical characteristic of the HPLC method

Linearity, detection limit, recovery and precision

Linearity of standard curve, detection limit, precision and recovery of method for determination of AA, TH and RB in RHN is shown in Table 1. An “R” value above 0.9989 was obtained for AA, TH and RB. Coefficients of determinations ($r^2$) were determined as 99.67%, 99.93% and 99.83% for AA, TH and RB, respectively. The detection limit for AA, TH and RB, based on “S/N” (signal/noise) of 3 (Li & Chen, 2000), were 0.1, 0.5 and 0.2 mg/L, respectively. The reliability of the method was confirmed by recovery experiments. Standard addition procedure was used for the recovery test. Thus, 50, 10 and 2 mg/L concentrations of AA, TH and RB standards were added to the samples, respectively. In each addition level, six determinations were realized. The average percentage recoveries of AA, TH and RB in rosehip nectar were found to be 95.8 ± 0.52%, 97.8 ± 0.45% and 98.6 ± 0.70%, respectively. 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 0.62% for AA, 2.84% for TH and 3.60% for RB in our study. In addition, the low RSD value also shows non-variability of the data.

3.2 Characteristics of RHN 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 solid, 3.85 ± 0.07 pH value, 0.35 ± 0.01% total acidity and 12.29 ± 0.02% total sugar. Soluble solids and pH value of rosehip nectar (glass-bottled) were reported as 16.2 Bx and 3.50, respectively (Duru et al., 2012).

3.3 Thermal degradation of AA, TH and RB in RHN

Thermal stability of AA, TH and RB in RHN were studied at 70, 80, 90 and 95 °C. Figure 2 shows the thermal degradation of AA, TH and RB of the RHN during heating period. Equation 2 was used for the determination of order of reaction. As seen from Figure 2, the degradations of AA, TH and RB are fitted to first-order kinetic model. As the heating temperature and time increased, the degradation of AA, TH and RB increased. A linear relation, which shows the plot for AA, TH and RB in RHN, correlates to first-order degradation kinetics with the correlation coefficient above 0.98 for all cases. It is pointed out that most quality-related reaction rates are either zero or first-order reactions and statistical differences between zero or first-order reactions may be insignificant (Labuza & Riboh 1982). On the other hand, there is no published data on the thermal degradation of AA, TH and RB in RHN. Therefore, we compare our results with the reported values from other sources.

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

Table 1. Linearity of standard curve, detection limit, recovery and precision of the proposed method for determination of AA, TH and RB in RHN.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Linear range (mg/L)</th>
<th>$R$</th>
<th>$r^2$</th>
<th>Detection limit (mg/L)</th>
<th>Initial content (mg/L)</th>
<th>Content after addition (mg/L)a</th>
<th>Recovery (%)</th>
<th>Mean SDa</th>
<th>Precision R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>5.0-250.0</td>
<td>0.9992</td>
<td>99.67</td>
<td>0.1</td>
<td>162.3 ± 1.1</td>
<td>210.8 ± 0.24</td>
<td>96.60 ± 0.52</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td>1.0-50.0</td>
<td>0.9996</td>
<td>99.93</td>
<td>0.5</td>
<td>25.2 ± 0.7</td>
<td>34.1 ± 0.16</td>
<td>97.16 ± 0.45</td>
<td>2.84</td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>1.0-40.0</td>
<td>0.9989</td>
<td>99.83</td>
<td>0.2</td>
<td>1.4 ± 0.5</td>
<td>3.30 ± 0.10</td>
<td>93.86 ± 0.70</td>
<td>3.60</td>
<td></td>
</tr>
</tbody>
</table>

a50 mg for ascorbic acid, 10 mg for thiamine and 2 mg for riboflavin; Mean ± standard deviation.
Earlier, Karhan et al., (2004) reported that ascorbic acid degradation under anaerobic circumstances in rosehip pulp follows the first-order kinetics at the temperature ranged from 70 to 95 °C. A first-order kinetics of thiamine degradation in red gram splits (*Cajanus cajan* L.) at pH 4.5, 5.5 and 6.5, over a temperature range of 50-120 °C (steady state temperature process) was observed by Rekhaa et al. (2004). In addition, first order reaction behavior of ascorbic acid, thiamine and other vitamin degradation in foods and model systems under different conditions by a number of researchers (Mulley et al., 1975; Vikram et al., 2005; Mauri et al., 2007; Peleg et al., 2016) also have been reported. On the other hand; there are no published data on the thermal degradation of TH and RB in RHN. So, we didn’t compare our results with the literature.

Table 2 shows the degradation rate constants (k), half-lives (t1/2), quotient indicator (Q10) and activation energies (Ea) for AA, TH and RB in RHN. The slope of the linearized plot of ln(C/C0) versus time was used in order to estimate the degradation rate constants. As the temperature is increased, the rate constants of AA, TH and RB also increased. This result suggests that degradations of AA, TH and RB are temperature-dependent. AA showed the lowest degradation rate constant, followed by TH and RB, suggesting that AA is less prone to thermal degradation when compared to TH and RB. The rate constant of AA 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 AA was very unstable at 95 °C as approved by higher k values resulting a fast degradation. In contrast, the rate constants of TH and RB increased significantly from 70 to 80 °C and increased slightly with the increment of temperature from 80 to 90 °C and 90 to 95 °C. The k values of AA, TH and RB varied between 4 x 10^{-3}, 7 x 10^{-3}, 18 x 10^{-3} and 7 x 10^{-3}, 18 x 10^{-3} over the temperature range of 70-95 °C, respectively (Table 2). Consequently, degradation of AA, TH and RB increased with the increment of heating temperature and time. AA is easily destroyed during processing and storage. In contrast to alkaline and neutral pH, the AA is more stable at acid pH (for example, in citrus juice) (Combs, 2008). There are several studies about the thermostability of riboflavin on different foods.

Table 2. Effect of temperature on the activation energy (Ea), reaction rate constant (k), Q10 and half-life (t1/2) values of AA, TH and RB in RHN.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>T (°C)</th>
<th>k 10^{3} (1/min)</th>
<th>t1/2 (min)</th>
<th>Q10 70-80 °C</th>
<th>Q10 80-90 °C</th>
<th>Q10 90-95 °C</th>
<th>Ea (kJ/mol)</th>
</tr>
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<tr>
<td>AA</td>
<td>70</td>
<td>4</td>
<td>173.29</td>
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<td></td>
<td>55.30</td>
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<td></td>
<td>80</td>
<td>8</td>
<td>86.64</td>
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<tr>
<td></td>
<td>90</td>
<td>14</td>
<td>49.51</td>
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<td></td>
<td>95</td>
<td>15</td>
<td>46.21</td>
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<tr>
<td>TH</td>
<td>70</td>
<td>7</td>
<td>99.02</td>
<td></td>
<td></td>
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<td>36.38</td>
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<td>95</td>
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<td>38.51</td>
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<td>RB</td>
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<td>77.02</td>
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<td>90</td>
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<td>95</td>
<td>18</td>
<td>38.51</td>
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</table>
Quotient indicator ($Q_{10}$) values of AA, TH and RB were calculated using the Equation 4. Table 2 shows the $Q_{10}$ values of AA, TH and RB in RHN over the temperature range of 70 to 95°C. Over the temperature range of 70 to 95°C, $Q_{10}$ values, for each 10°C increment were different in rosehip nectar. The highest $Q_{10}$ values for AA, TH and RB are obtained within the ranges of 70 to 80 °C, 80 to 90 °C and 80 to 90 °C, respectively. This result indicates that in these ranges the degradation kinetic was strongly affected by the temperature. In the range of 90 to 95 °C, the $Q_{10}$ values of AA, TH and RB are very close to each other and lower in comparison to other ranges, indicating that within this range the degradation kinetics are very few affected by the temperature change. In addition to all this information, no comparison were made with the literature. Because there is no published scientific data on the topic. For this reason, the calculated $Q_{10}$ values of AA, TH and RB are compared to the literature reported $Q_{10}$ values of other sources. $Q_{10}$ value of AA in the rosehip pulp during heating under anaerobic circumstances was reported as 1.21 (Karhan et al., 2004).

4 Conclusions

Degradation of AA, TH and RB 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 AA, TH and RB of RHN for long term heating of rosehip nectar was 70 °C. The RHN producing industry may benefit from the results of this study when controlling the amount of examined three water-soluble vitamins in production process. The results of this study will help the RHN producing industry when controlling the amount of AA, TH and RB. These parameters are important quality criterias for RHN. Therefore, knowing the degradation kinetics of AA, TH and RB are important. To the best of our knowledge, there is no published study on the thermal degradation of AA, TH and RB of RHN.

Acknowledgements

This work was supported by research Grant 2011FBEO78 from Research Project Fund (BAP) of Pamukkale University. The authors thank the GUMUSSU FOOD A.S (Yüksel DEMIR) for providing the rosehip.

References


