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Araştırma Makalesi

Effect of Ripening Stages and Oven Drying on the Carotenoid Composition of Goji Berry (*Lycium barbarum* L.) Fruits[&]

Pınar ŞENGÜN[1], Çetin KADAKAL[1]*

[1]Pamukkale Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Denizli
*Sorumlu Yazar: ckadakal@pau.edu.tr

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Abstract

In this study firstly, pH, titratable acidity, water soluble solid (°Bx) and carotenoid content of goji berry fruits in three different ripening stages (mature green, intermediately ripe, fully ripe) were determined. Secondly, the fully ripe goji berry fruits were dried at 50, 60 and 70 °C in an oven drying and the changes in carotenoid composition and kinetic parameters were investigated. Thermal degradation of carotenoids in goji berry fruit fitted to the first order reaction model and k, $t_{1/2}$, Q_{10} and E_a values were calculated. Changes in the carotenoid composition were observed as a result of the ripening of the fruit. Lutein was found as the predominant carotenoid at mature green goji berry fruit, while zeaxanthin dipalmitate was at intermediately and fully ripe goji berry. Chlorophyll a and b were detected in the mature green fruit but not in the intermediately and full ripe fruit. The results demonstrated that drying temperature is an important factor affecting carotenoid degradation in goji berry fruits and different ripening stages have important effect on the carotenoid content of goji berry.

Key words: Carotenoid, drying, goji berry, HPLC, ripening.

Goji Berry (*Lycium barbarum* L.) Meyvelerinin Olgunlaşma Aşamalarının ve Fırında Kurutmanın Karotenoid Bileşimi Üzerine Etkisi

Öz

Bu çalışmada ilk olarak, goji berry meyvelerinin üç farklı olgunlaşma evresinde (olgun yeşil, orta olgun, tam olgun) pH, titrasyon asitliği, suda çözünür kurumadde (°Bx) ve karotenoid içeriği belirlenmiştir. İkinci olarak, 50, 60 ve 70 °C'de kurutulan tam olgunlaşmış meyvelerin karotenoid bileşimi ve kinetik parametrelerdeki değişimler incelenmiştir. Goji berry meyvesindeki karotenoidlerin termal bozunması birinci dereceden reaksiyon modeline uymuş ve k, $t_{1/2}$, Q_{10} ve Ea değerleri hesaplanmıştır. Meyvenin olgunlaşmasına bağlı olarak karotenoid bileşiminde değişiklikler gözlemlenmiştir. Lutein, olgun yeşil goji berry meyvesinde baskın karotenoid olarak bulunurken, zeaksantin dipalmitat orta ve tamamen olgun goji berry meyvesinde tespit edilmiştir. Olgun yeşil meyvelerde klorofil a ve b tespit edilmesine ragmen, orta ve tam olgun meyvelerde tespit edilememiştir. Sonuç olarak, goji berry meyvelerinde kurutma sıcaklığının karotenoid bozunmasını etkileyen önemli bir faktör olduğu ve farklı olgunlaşma aşamalarının goji berry meyvesinin karotenoid içeriği üzerinde önemli bir etkiye sahip olduğu ortaya çıkmıştır.

Anahtar kelimeler: Karotenoid, kurutma, goji berry, HPLC, olgunlaşma.

Introduction

Goji berry (*Lycium barbarum* L.), belonging to Solanecea family, has been grown for 2500 years and has more than 70 cultivars in different region (Kulczyński and Gramza-Michałowska, 2016). Most of the commercially produced goji berry is being grown in China, Middle East, Mongolia, Japan, Tavy and Himalayan (Amagase and Nance, 2009; Maughan, 2015). Goji berry, which is called with various names such as kurt

üzümü, super fruit, hedge plant and Gouqizi in different cultures, have become popular in recent years due to their rich nutritional value, antioxidant properties and health benefits (Potterat, 2010; Shahrajabian et al., 2020).

Goji berry fruit which has the taste between blueberry and cherry is juicy and sweet (Amagase and Farnsworth, 2011). This fruit is generally consumed in China as fresh, dried, herbal tea or by being added to Chinese soups, meat and vegetarian dishes. It is also used in fruit juice, wine and tonic production (Donno et al., 2015). Apart from the fruits of the goji plant, its leaves are also consumed as tea (Bruno, 2009).

Goji berry contains macronutrients that provide a high portion of daily expenditure. It contains 68% carbohydrate, 12% protein, 10% fiber and 10% lipid. It is also rich in vitamins and minerals. The high antioxidant activity of the fruit is due to the carotenoids and phenolic compounds it contains. The attractive red-orange color of the fruit is due to carotenoids (Endes et al., 2015; Islam et al., 2017; Skenderidis et al., 2019).

The carotenoid composition of goji berry fruit includes zeaxanthin, violaxanthin, neoxanthin, β-carotene, β-cryptoxanthin and lutein. Zeaxanthin is found in very high concentrations in goji berries. Antheraxanthin, lutein, violaxanthin, neoxanthin and β-carotene have been reported in the green form of the fruit (Inbaraj et al., 2008; Karioti et al., 2014; Liu et al., 2014; Hempel et al., 2017; Patsilinakos et al., 2018).

As a result of clinical studies, it has been reported that patients who consume goji berry fruit have a decrease in psychological and neurological characteristics, joint and gastrointestinal system problems, muscle functions, diabetes, ligament pain, depression, sleep quality, fatigue, impaired concentration, memory loss and shortness of breath (Chang and But, 2001; Amagase and Norman, 2011; Potterah, 2010; Wenli and Shahrajabian, 2019; Szot et al., 2020).

Research on goji berry fruits has mainly focused on chemical components, polysaccharides, fatty acids, carotenoid contents, mineral substance contents, biological activities, health benefits and traditional uses of the fruits (Inbaraj et al., 2008; Potterah, 2010; Amagase and Farnsworth, 2011; Jin et al., 2013; Kulczyński and Gramza-Michałowska, 2016; Koçyiğit and Şanlıer, 2017; Shah et al., 2019).

The first aim of the study; to determine the chemical (pH, titratable acidity, dry matter, water-soluble matter and carotenoid composition) properties of goji berry fruits grown in Denizli region, the changes in carotenoid content in three different ripening stages (mature green, intermediately ripe, fully ripe). The second aim is to determine the kinetic parameters of the carotenoid (order of reaction, reaction rate constant (k), half-life (t1/2)activation energy (Ea) and Q10) to determine the changes in the carotenoid content of ripe fruits dried in the drying oven at 50, 60 and 70 °C.

Materials and Methods

Materials

The NQ1 variety goji berry fruits (*Lycium Barbarum* L.) were used as material and hand-harvested (during June-August in 2019) from the orchard of Redlife Company in Çivril district of Denizli province of Turkey. The fruits were harvested at three different maturity stages (mature green, intermediately ripe, fully ripe). Ten different plants were used for the collection of fruits from different ripening stages. The analysis of the samples started after the samples reached to the laboratory.

Drying procedure

The samples were dried in a tray drying cabinet (Yücebaş Makine Tic. Ltd. Şti., İzmir, Turkey) until 13-15% water content. Dryer comprised an electric heater, a centrifugal fan to provide airflow and an electronic proportional controller (EUC442 model, ENDA, Turkey). The dryer's internal size was 70 cm × 55 cm × 100 cm, the range of workable relative humidity was 20%–95% and the range of workable temperature was 40–120°C. The samples were dried with heated air at the temperatures of 50, 60, 70 °C. The cabinet was heated for 1 hour before the start of drying process to reach a constant temperature. The flow rate of the drying oven was adjusted to 0.2 m s^{-1} and its relative humidity to 20%.

Extraction of Carotenoid

The carotenoid composition (β-cryptoxanthin palmitate, zeaxanthin, zeaxanthin dipalmitate, lutein, violaxanthin, antheraxanthin, neoxanthin, β-carotene and chlorophyll composition (chlorophyll a and chlorophyll b) of the goji berry samples was analyzed using HPLC (High Performance Liquid Chromatography). Method suggested by Sadler et al. (1990), Gama and Sylos (2005) and Cemeroğlu (2007) was used for carotenoids extraction with a slight modification. 5 g of goji berry sample was weighed and mixed with a solution of hexane: methanol: acetone (50:25:25) containing 0.1% butylated hydroxytoluene (BHT), then 5 mL of ultrapure water was added. The samples were homogenized

with a blender (Waring, USA) and were centrifuged (Core NF800, Turkey) at 6000 rpm for 10 min at 4 °C. This process was repeated until the colorless phase remained in the samples. After centrifugation, the colored supernatant containing carotenoids was separated into a tube and this process was repeated until the colorless phase remained in the sample. Then the solvents of the supernatant were removed in a rotary evaporator (CLS Scientific, Turkey) at 40 °C. The residue was dissolved by adding 2 mL of tetrahydrofuran: methanol (1: 9 v/v) onto the remaining residue. The residue obtained was filtered through a 0.45 µm PTFE type filter (Sartorius, Germany) and kept at -20 ° C until analyzed in HPLC device. The study was carried out in 2 parallel for each sample.

HPLC conditions

The HPLC system (Shimadzu Corporation, Japan) consisting of a UV-VIS DAD detector set at 450 nm, a quadruple liquid chromatography pump (LC20AD, Shimadzu), a degasser (DGU-20A3, Shimadzu) a column oven (CTO-20A, Shimadzu) and nucleosil based C18 column (250 x 4.6 mm, ID, Macherey-Nagel) was used for the analysis. The data were viewed with the program of Shimadzu Software. Mobile phase was gradient and the conditions were; [0-25 min acetonitrile:methanol:ethyl acetate (99:1:0), 25-55 min acetonitrile: ethyl acetate: methanol: (60:30:10), and 55-60 min acetonitrile:methanol:ethyl acetate (99:1:0).

Kinetic Parameters

General equation explained for thermal degradation kinetics is as given bellow (Equation 1) (Labuza,1985);

$$\frac{dC}{dt} = k[C]^m \qquad (1)$$

Where;

C: The concentration of the compound at the specified time (µg g $^{-1}$),
k: reaction rate constant (h^{-1}),
m: order of reaction,
t: time
When Equation 1 is integrated and *m* equals to one, the equation is calculated given below (Equation 2):

$$lnC = lnC_0 - kt \qquad (2)$$

Where;
ln C: natural logarithm of the residual carotenoids
ln C_0: initial content of carotenoids
k: rate constant (h^{-1});
t: time

The temperature dependence of carotenoids could be calculated with Equation 3:

$$k = k_0\, x\, e^{-\frac{Ea}{RT}} \qquad (3)$$

When Equation 3 is regulated, Equation 4 is written as follows:

$$lnk = \left(-\frac{Ea}{R}\right) x \left(\frac{1}{T}\right) + lnk_0 \qquad (4)$$

Where;
k: reaction rate constant (h^{-1})
k_0: frequency factor (h^{-1})
R: universal gas constant (1.987 x 10^{-3} kcal mol^{-1} K^{-1} or 8.314 x 10^{-3} kJ mol^{-1} K^{-1})
Ea: activation energy (kcal mol^{-1} or kJ mol^{-1})
T: absolute temperature (K)

Quotient indicator (Q_{10}) describe the dependence of the reaction rate on temperature and Equation 5 is used for the calculation:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\left(10/T_2 - T_1\right)} \qquad (5)$$

Where, k_1 and k_2 are the rate constants of carotenoids degradation at temperatures T_1 and T_2, respectively.

The time required to halve the concentration (half-life time) is calculated using Equation 6:

$$t_{1/2} = -\ln(0.5)\, xk^{-1} = 0{,}693xk^{-1} \qquad (6)$$

Further Determinations

Methods, suggested by AOAC (1990), were used for the analysis of pH, titratable acidity and water soluble solid (°Bx). Initially, goji berry berries were separated from their stems and crushed in a blender (Waring, ABD). Then it was filtered through filter paper and goji berry juice was obtained. Goji berry juice was used for titration acidity, pH and brix analysis. A glass electrode tipped pH meter (PL-700PV, Gondo-Taiwan) was used to measure the pH value of goji berry juice. For titration acidity analysis, 20 ml of distilled water was added to 10 ml of goji berry juice and then titrated with 0.1 N NaOH until the pH value was 8.1. The NaOH consumption was determined and the titration acidity was calculated in terms of tartaric acid. Brix analysis was performed with a digital refractometer (Milwaukee MA871 Refractometer, Europe).

Color analysis was performed using Hunter Lab Color Miniscan XE (45/0-L, USA). Goji berry berries were placed on a white background

and the measurement was taken by closing a transparent glass. Results are given in terms of L*, a* and b*. L* represents lightness/darkness, a* represents redness/greenness and b* represents yellowness/blueness.

Statistical analysis

For statistical analysis, SPSS 22.0 software (IBM Corporation, Armonk, NY) was used and results were expressed to mean ± standard deviation (SD). Differences between groups were specified with the Duncan test. ANOVA was used to assess differences between treatments with the significance level (P = 0.05).

Result and Discussion

Characteristics of Goji berry fruits

The change in the total soluble solid, titratable acidity and pH values of goji berries in different ripening stages was shown in Table 1. As seen in Table 1, the amount of water-soluble solids increased with the advancement of ripening. Zhang et al. (2016) and Jatoi et al. (2017) have found the water soluble solid of goji berry fruit between 14.7-19.3% and 16.43-18.90%, respectively. The pH value decreased from the mature green to the intermediately ripe and increased in the fully ripe. Çolak et al. (2016) and Donno et al. (2017) determined the pH value of goji berry fruit as 3.25-4.36 and 3.80, respectively. Titratable acidity was determined at the highest in intermediately ripening tage. Çolak et al. (2016) determined the titratable acidity of goji berry fruits in the range of 0.9-1.5%.

Table 1. Changes in water soluble solid, pH and titratable acidity of goji berry fruits in different ripening stages.

Ripening Stages	Water soluble solid (%)	pH	Titratable acidity (%)
Mature green	7.3±0.04^{c*}	5.38±0.02^{a}	0.46±0.05^{a}
Intermediately ripe	11.5±0.01^{b}	5.07±0.01^{c}	0.49±0.02^{b}
Fully ripe	18.6±0.03^{a}	5.21±0.01^{b}	0.58±0.04^{c}

*Values within a column followed by different letters are significant (P <0.05)

Changes in carotenoid content in different ripening stages of goji berry fruit

Changes in the carotenoid and chlorophyll content of goji berry fruit collected in three different ripening stage was presented in Table 2. Lutein has been identified as the predominant carotenoid in mature green goji berry fruit, while zeaxanthin dipalmitate has been identified as the predominant carotenoid in intermediately and fully ripe goji berry fruit. Chlorophyll a and b were detected in the mature green fruit but could not be detected in the intermediately and full ripe fruit. Hempel et al. (2017) stated that they detected β-carotene, lutein, violaxanthin, zeaxanthin, antheraxanthin and neoxanthin in the mature green, and zeaxanthin, zeaxanthin dipalmitate, β-cryptoxanthin palmitate and antheraxanthin dipalmitate and β-carotene in fully ripe goji berry fruit.

Liu et al. (2014) have investigated the carotenoid content depending on ripening of goji berry fruit, and they have found that the total carotenoid amount in the green mature fruit was 34.46 µg g^{-1} and 508.9 µg g^{-1} in the fully ripe fruit. They also reported a decrease in the amount of chloroplastic carotenoids such as lutein, violaxanthin and β-carotene depending on the ripening of the fruit.

Table 2 Changes in carotenoid and chlorophyll content (µg g^{-1}) of goji berry fruit at different ripening stages.

Carotenoid	Mature green	Intermediately ripe	Fully ripe
Lutein	96.93±0.86^{a*}	33.97±0.18^{b}	ND**
Zeaxanthin	19.45±0.09^{a}	7.27±0.07^{b}	1.48±0.06^{c}
β-cryptoxanthin palmitate	ND	0.60±0.03^{b}	1.83±0.04^{a}
Zeaxanthin dipalmitate	ND	60.02±1.11^{b}	220.90±1.70^{a}
β-carotene	48.89±0.19^{a}	18.31±0.13^{b}	2.26±0,021^{c}
Violaxanthin	34.28±0,17^{a}	12.92±0,09^{b}	ND
Antheraxanthin	6.85±0,07^{a}	6.85±0.06^{a}	ND
Neoxanthin	13.50±0.10^{a}	3.84±0,08^{b}	ND
Chlorophyll a	2.15±0.04	ND	ND
Chlorophyll b	1.82±0.11	ND	ND

*Values within a line followed by different letters are significant (P <0.05), **ND; Not detected.

Changes in the carotenoid content of goji berry fruit during drying

The changes in carotenoid content as a result of drying the fully ripe goji berry fruit in a drying oven at 50, 60 and 70 ° C are given in Table 3. Drying processes for 50, 60 and 70 ° C were completed in 22, 17 and 9 hours, respectively. As a result of the drying process at 50, 60 and 70 ° C temperatures, losses were observed in the carotenoids in the goji berry fruit. Fratianni et al. (2018) dried goji berry fruit at three different temperatures, and determined that values of Zeaxanthin, zeaxanthin dipalmitate, lutein, β-cryptoxanthin, β-carotene and total carotenoids were decreased after drying process. Ma et al. (2008) dried the goji berry fruit and reported decreases in the amount of zeaxanthin dipalmitate and total carotenoids at the end of the drying process.

Table 3 Changes in the carotenoid content (μg g^{-1}) of goji berry fruit during oven drying at 50, 60 and 70 °C.

Drying temperature °C	Time (h)	Carotenoids			
		β-cryptoxanthin palmitate	Zeaxanthin	Zeaxanthin dipalmitate	β-carotene
50°C	0	1.83±0.04^{a*}	1.48±0.02^{a}	220.9±1.70^{a}	2.26±0,021^{a}
	5	1.66±0.03^{b}	1.47±0.01^{a}	193.5±1.55^{b}	2.25±0.01^{a}
	10	1.59±0.01^{c}	1.45±0.05^{a}	184.6±0.42^{c}	2.24±0.04^{a}
	15	1.55±0.01^{d}	1.41±0.05^{b}	179.9±0.42^{d}	2.21±0.04^{b}
	20	1.52±0.07^{e}	1.39±0.02^{b}	177.7±0.56^{de}	2.20±0.28^{b}
	22	1.51±0.01^{e}	1.38±0.02^{b}	175.05±0.35^{e}	2.19±0.01^{b}
60°C	0	1.83±0.04^{a}	1.48±0.02^{a}	220.9±1.70^{a}	2.26±0.021^{a}
	5	1.59±0.01^{b}	1.46±0.02^{a}	198.6±1.48^{b}	2.23±0.05^{a}
	10	1.58±0.04^{b}	1.41±0.01^{b}	192.3±1.06^{c}	2.20±0.08^{ab}
	15	1.47±0.02^{c}	1.38±0.02^{bc}	186.2±1.55^{d}	2.17±0.042^{b}
	17	1.43±0.01^{c}	1.36±0.01^{c}	181.1±0.98^{e}	2.16±0.028^{b}
70°C	0	1.83±0.04^{a}	1.48±0.02^{a}	220.9±1.70^{a}	2.26±0.021^{a}
	3	1.47±0.03^{b}	1.44±0.01^{b}	206.3±1.28^{b}	2.22±0.01^{ab}
	6	1.37±0.03^{c}	1.39±0.02^{c}	195.7±0.84^{c}	2.18±0.08^{b}
	9	1.35±0.02c	1.34±0.05d	193.1±0.42c	2.15±0.04b

*Values within a column followed by different letters are significant (P <0.05).

Thermal degradation kinetics of carotenoids

Thermal degradation of carotenoids in goji berry fruit was studied at 50, 60 and 70 °C. As seen from Figure 1, thermal degradation of carotenoids was fitted to first order reaction model. The k, $t_{1/2}$, Q_{10} and E_a values of carotenoids was presented in Table 4.

The k value of carotenoids increased with the increasing temperature. β-carotene showed the lowest degradation rate constant, followed by zeaxanthin, zeaxanthin dipalmitate and β-cryptoxanthin palmitate. The highest rate constants were calculated at 70 °C.

Half-life time decreased as temperature increased, so carotenoids have degraded faster at higher temperatures. The highest thermal stability for carotenoids was calculated as 50 °C in goji berry fruits. Demiray et al. (2013) stated that rate constant of lycopene and β-carotene increased and $t_{1/2}$ decreased with the increment in drying temperature in tomatoes drying process. Kadakal et al. (2017) stated an increase for the degradation rate constant and a decrease for the half-life values depending on the temperature increase in rosehip nectar. The Q_{10} values was calculated at temperatures ranging from 50 to 70 °C. The highest Q_{10} value was calculated in the range of 60-70 °C.

Higher Q_{10} value indicates that the decomposition reaction will be more affected by the temperature change. Demiray et al. (2013) found the minimum and maximum Q_{10} values of lycopene at 60-70 °C and 70-80 °C, respectively, in a study where tomatoes were dried at different drying temperatures.

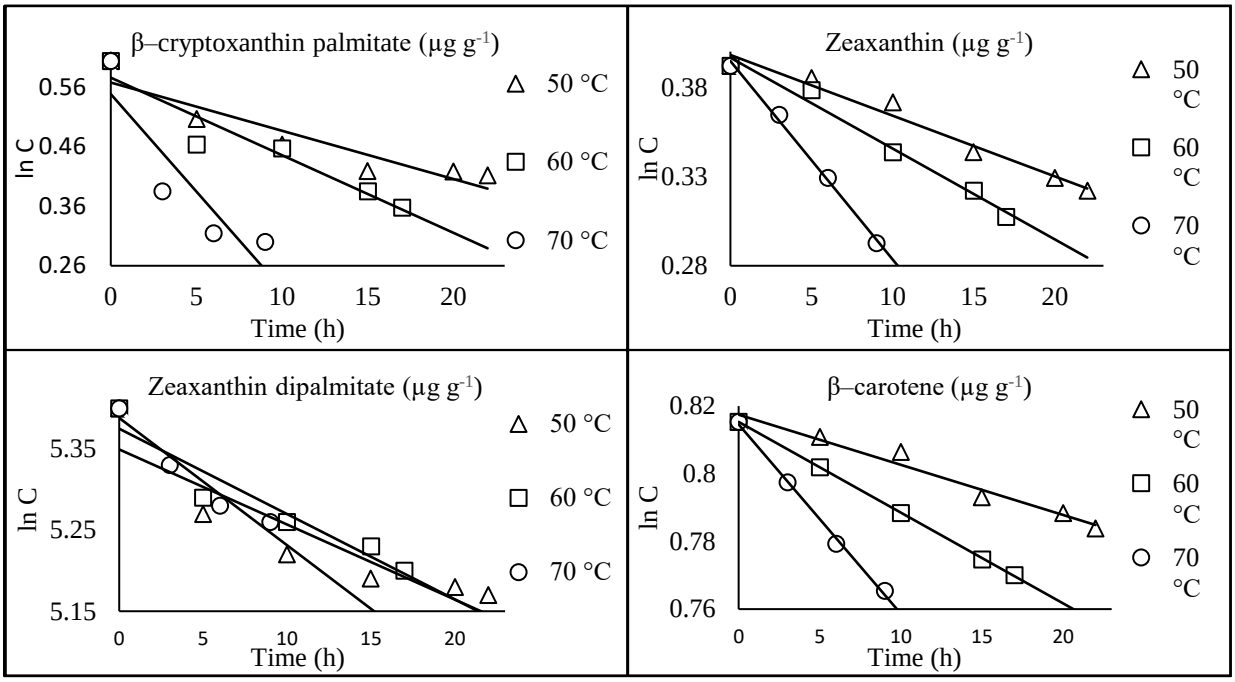

Figure 1 First order thermal degradation of β-cryptoxanthin palmitate, zeaxanthin, zeaxanthin dipalmitate and β-carotene in goji berry at three different temperatures.

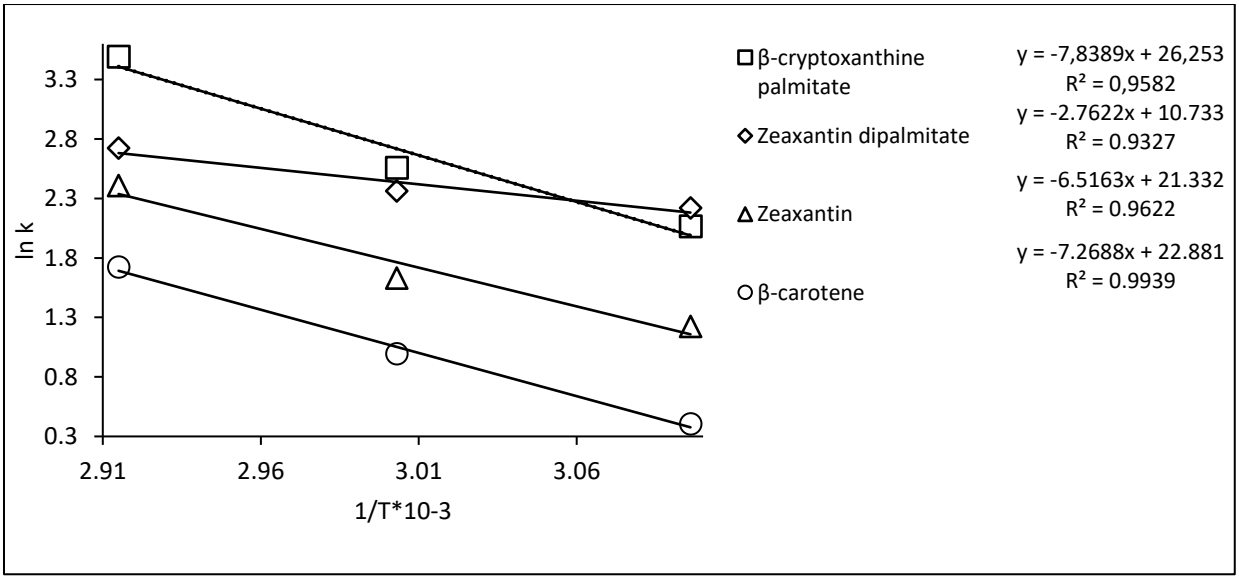

Figure 2 Arrhenius plots for carotenoids thermal degradation in goji berry fruits at different temperatures during drying.

As seen from Table 4, the highest E_a value was found in β-cryptoxanthin palmitate as 15,57 kcal.mol^{-1}, and E_a values of zeaxanthin dipalmitate were calculated higher than zeaxanthin. When Q_{10} values of all carotenoids in our study were compared, it was understood that β-cryptoxanthin and zeaxanthin are more sensitive to increase in temperature (50-60 °C). Arrhenius plots for carotenoids thermal degradation in goji berry fruits at different temperatures during drying is given in Figure 2.

Table 4 Kinetic parameters [(reaction rate constant (k), activation energy E_a, Quotient indicator Q_{10} and half-life $t_{1/2}$)] of carotenoids in goji berry fruits during oven drying at 50, 60 and 70 °C.

Carotenoids	T (°C)	k x 10^{-3} (h^{-1})	$t_{1/2}$ (h)	Q_{10}		E_a (kcal mol^{-1})	E_a (kJ mol^{-1})
				50-60 °C	60-70 °C		
β-cryptoxanthin palmitate	50	7.9	87.7	1.63	2.54	15.57	65.17
	60	12.9	53.7				
	70	32.8	21.1				
Zeaxanthin	50	3.4	203.8	1.50	2.17	5.48	22.96
	60	5.1	135.8				
	70	11.1	62.4				
Zeaxanthin dipalmitate	50	9.2	75.3	1.15	1.43	12.94	54.17
	60	10.6	65.3				
	70	15.2	45.5				
β-carotene	50	1.5	462.1	1.80	2.07	14.42	60.43
	60	2.7	256.6				
	70	5.6	123.7				

Conclusion

The goji berry fruit has physically and biochemically changed during ripening. The drying process was completed at 50-70 °C for 22-9 hours. With the drying process, decreases were observed in the concentration of carotenoids. The drying speed and drying time were affected by drying temperature. The degradation of the carotenoids in the goji berry fruit after drying in the drying cabinet fits the first order kinetic model. As the drying temperature increased, degradation rate of carotenoids increased and $t_{1/2}$ decreased. The carotenoid with the highest thermal stability was zeaxanthin with the lowest E_a value. β-cryptoxanthin and zeaxanthin are more sensitive to increase in temperature because of the highest Q_{10} (60-70 °C) values. The best temperature for the less degradation of carotenoids for drying of goji berry fruits was 50 °C. In terms of the future research planning, studies should be carried out on more efficient drying methods that may cause less carotenoid degradation.

&: This study was produced from the master's thesis titled "Changes in carotenoid composition on goji berry (*Lycium barbarum* L.) fruits during ripening stages and drying process".

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Researchers' Contribution Rate Statement Summary: The authors declare that they have contributed equally to the manuscript.

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