DEGRADATION KINETIC OF *TRANS*-RESVERATROL IN SEEDLESS SULTANA AND ÇALKARASI GRAPES DURING TRAY DRYING

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Abstract

In this study, pH, brix, total acidity and trans-resveratrol properties of two different grapes (seedless Sultana and Çalkarası), dried at various temperatures and periods (solar and tray dryer), were investigated. The content of trans-resveratrol was detected by using high performance liquid chromatographic (HPLC) method.

During tray drying, degradation of trans-resveratrol in seedless Sultana and Çalkarası grape varieties was fitted to a first- order reaction kinetic model. Arrhenius relationship was used in order to describe the temperature dependence of reaction. Activation energies for trans-resveratrol in seedless Sultana and Çalkarası grape varieties between 50 to 65 °C were found to be 4.97 and 1.63 kcal/mol, respectively. In addition, other kinetic parameters such as reaction rate constant (k), quotient indicator (Q_{10}) and half-life ($t_{1/2}$) values were also determined.

Key words: *drying, grape, HPLC, trans-resveratrol*

1. INTRODUCTION

Viniculture is one of the most common agricultural activities in the world. The history of viniculture dates back to BC 3000 in the region called little Asia, including Anatolia (Kaya 1995). Vine is cultivated in temperate climate zone and compared to other plants, it involves nearly the most variety. It is estimated that there is kind of vine more than 10.000 (Göktaş 2008).

The cultivation of grape is very widespread because of its poor selectivity in terms of soil and climate, easiness of growth and much different ways of consumption (Akova 2009). Grape is also one of the most common grown fruit in Turkey due to the same reasons.

Grape and its products are good source of food because of its different flavours, nutritional value and versatile influences on human health (Çelik et al. 1998). Grape contains high level of sugar and thus, it is good source of calorie. In addition, it is considered as important product in terms of mineral matters such as calcium, potassium, sodium and iron and some vitamins (A, B_1, B_2, B_3 and C). The composition of grape mainly consists of 81 g water, 17.3 g carbohydrates and the rest is low amount of protein, lipid and ash. However, dried grape is rich fruit with high level of carbohydrate and protein contents. In addition, polyphenols such as anthocyanins, tannins, antioxidants such as ellagic acid and resveratrol are also present grapes (Türkben 2000).

Grape and grape products are not only important in terms of nutritional value but also have great significance due to high positive effect on human health. It is known that grape cures rheumatism and arthralgia, regulates cardiovascular system, strengthens immune system, prevents liver diseases, cleans up blood and renews cells particularly when consumed with skins and seeds. Furthermore, it prevents occurrence of inflammation in the event of allergy and calcification and protects osteoporosis caused by menopause thanks to ellegig acid (Aras 2006). Nevertheless, these important effects differ considerably based on process techniques (Çelik et al. 1998).

Polyphenols are one of the most abundant compounds in grapes and 8%, 46-69% and 12-50% of these phenolics are present in pulp, seed and skin, respectively. The main phenolic compounds in grapes are anthocyanins, catechins, epicatechins and resveratrols (particularly in seeds) (Uluocak 2010).

Phenolic compounds are separated into two groups as phenolic acids and flavonoids. Flavonoids are polyphenolic antioxidants found in natural herbal teas, fruits and vegetables (Nizamlıoğlu & Nas

2010). 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 (Gökalp et al. 2002; Cvejic et al. 2017). Phenolics have antioxidant, antimutagenic and anticarcinogenic effects and are capable of altering gene expression (Nakamura et al. 2003; Ercisli 2007).

Resveratrol (trans-3,5,4'-trihydroxystilbene), a phenolic substance located in several parts of the grapes, is mainly present in the skin. Besides, resveratrol can be a constituent of woody part of the plant roots, grapeseeds or stalks, and also an induced substance, as a resistance factor to fungi such as *Botrytis cinerea* and *Plasmopara viticola* (Celotti et al. 1996). In nature resveratrol has two forms as *trans* and *cis* (Abril et al. 2005). Resveratrol is a strong antioxidant that blocks cell inflammation caused by free radicals (Keskin et al. 2009). It is considered that *trans*-resveratrol can be responsible for the decrease in coronary heart disease observed among wine drinkers (Romero-Perez et al. 1996).

Due to significance of *trans*-resveratrol which is a strong antioxidant in grapes, it is important to calculate kinetics changes of *trans*-resveratrol during drying. This study aimed a) to present kinetic changes of *trans*-resveratrol during tray drying and b) to determine the effect solar drying on *trans*-resveratrol.

2. MATERIAL AND METHODS

2.1. Fruits

Recently, *Vitis Vinifera L.*, as known for seedless Sultana, has constituted 95% of dried grapes in the world markets (Williamson & Carughi 2010). It was reported that seedless Sultana is a reclaimed kind of round, seedless, dried grape at the end of 18th century (Otağ 2015). The other investigated kind of grape in this study is called Çalkarası. It is widely cultivated in region Denizli, a city in Turkey, and generally processed to rose wine due to its low colour potential (Darıcı 2011).

Grapes (Seedless Sultana and Çalkarası) were provided from Selcen village near Çal district in Denizli. Fresh fruits were transferred to department of Food Engineering at Pamukkale University and stored in polyethylene bags at 4°C in refrigerator until the time of analysis). All of grape varieties were grown in vineyards at 870 m above sea level in the Çal area, location in the province of Denizli (Western Turkey). The area is characterized by a favourable climate for grape cultivation. The sampling of all varieties was performed using the same procedure by randomly picking clusters from the top, central and bottom part of plant. Çalkaras and Sultana samples were collected from 13 (2.3 ha) and 15-year old vineyards (3.1 ha), respectively. The distance between the rows was 1.5 m and the distances between the grape vine canes 2.0 m. 5 kilograms of the grapes was collected from 40 to 50 plants for each variety. Two hundred berries per experimental unit were randomly collected from within clusters.

2.2. Selection of temperatures and tray drying periods

Because of the drying temperature range is 50-65 ºC for grapes in Turkey, four different temperatures (50, 55, 60 and 65 ºC) were selected in this study. The selection of the drying time of 72 hours is based on the fact that the moisture content reaches 16-18% at 65 °C.

2.3. Drying procedure

The samples were dried in two forms as solar and tray drying until 16-18% water content. The samples in tray drying were spread on a tray in the amount of 5-6 kg and dried with heated air at the temperatures of 50, 55, 60, 65 °C. Air flow rate and relative humidity on the cabinet dryer were adjusted to 0.2 m/s and 20%, respectively. The size of trays was 40x60 cm and the trays were made of stainless steel string in form of sieve. For solar drying 3-4 kg samples were used. Sampling in both drying process was carried out every 12 hours at regular intervals.

2.4. Determination of trans-resveratrol

Trans-resveratrol (Catalog No: 554325) standard was purchased from company Merck Milipore (KGaA, Darmstadt, Germany). Five different concentrations of *trans*-resveratrol standard prepared in methanol were used for the preparation of calibration curve. Five point calibration curve, covering the range of 1-500 ppb with the correlation coefficients of 0.999 based on the concentration (mμ/L) versus peak area (mAU), was prepared by using stock-standard solutions of *trans*-resveratrol.

2.5. Sample Preparation

Method, suggested by Jeandet et al. (1991), was used with slight modifications. 10g grape sample was added into methanol-water solution (90:10, v/v) and homogenized in laboratory blender. The extracts were centrifuged at 9000 rpm (Model 2-16, Sigma Bioblock Scientific) during 10 minutes and filtered. The extracts were stored at -20°C until the analysis. Following the filtering of extract using membrane filter (0.45 μm pore size, Schleicher & Schuell, Darmstadt-Germany), injected into HPLC apparatus.

2.6. Apparatus for HPLC

A liquid chromatography (Shimadzu Corporation, Kyoto, Japan) system consisting of a UV VIS DAD detector (Shimadzu 20AD UV Vis) set at 310 nm for the determination of *trans*-resveratrol was used to achieve the column eluate monitoring, a column oven (Shimadzu CTO-20A) set at 40 ºC, a quadruple liquid chromatography pump (LC20AD, Shimadzu), a degasser (SHIMADZU, DGU-20A3) and a Shimadzu Software Program was used for the analysis. Additionally, Bio Rad Aminex HPX-87H ion exclusion column (300×7.8mm2) was used in the HPLC system. Mobile phase, used in the HPLC system, was gradient and 2.5% acetic acid (Pump A) and 90% acetonitrile (Pump B) with the flow rate of 0.6 ml/min.

2.7. Recovery of trans-resveratrol

Grape samples containing known amounts of *trans*-resveratrol were spiked with the five additional levels of standard *trans*-resveratrol to determine the recovery. For this purpose, three determinations were carried out for each addition level.

2.8. Statistical analysis

SAS® software 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 ($P<0.05$) (SAS® 1985).

2.9. Calculation of kinetic parameters

A general equation for thermal degradation kinetics can be written as given below (Labuza 1982):

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

Where,

C : quantitative amount (μg/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:

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

Where, $[C]_0$ and $[C]_t$ are the initial and residual *trans*-resveratrol, respectively. k is the reaction rate constant (1/hour) and t represents the time (hour). Temperature dependence of *trans*-resveratrol was described by the Arrhenius equation 3, given below:

$$k = k_0 \times e^{-E_a/RT} \quad (3)$$

Where, k is the rate constant (milligrams *trans*-resveratrol per hour), k_0 the frequency factor (per min), Ea the activation energy (kilocalorie per mole), T the absolute temperature (K), and R is the universal gas constant (1.987 x 10^{-3} kcal/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:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}}{}_0 \quad (4)$$

Where, T is temperature in C, k_1 and k_2 are the rate constants of *trans*-resveratrol degradation at temperatures T_1 and T_2, respectively. The time required degrading 50% of original concentrations of *trans*-resveratrol was calculated using the equation 5 given below:

$$t_{1/2} = 0.693/k \quad (5)$$

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

2.10. Further Determinations

The analyses of water soluble solid (Bx), pH and total acidity (dry tartaric acid) were performed according to methods suggested by Cemeroğlu (1992).

3. RESULTS AND DISCUSSION

3.1. Characteristics of seedless Sultana and Çalkarası samples used

The characteristics of seedless Sultana and Çalkarası used in the study were as follows: 23.28±0.04 and 22.16±0.07 Bx soluble solids, 3.81±0.05 and 3.84±0.08 pH value, 5.60±0.02 (g/L) and 6.40±0.09 (g/L) total acidity, respectively. Minimum and optimum brix values of grapes for efficient drying are recommended as 19-20 and 22-23, respectively (Arzani et al. 2009).

Total soluble solids (°Brix) of seedless Sultana and Çalkarası grapes increased during solar and tray drying. The highest brix value was found (68.46) in seedless Sultana. Brix values were found to be 55, 64.2, 65 and 67.2 in seedless Sultana grapes which were dried at 50, 55, 60 and 65 C at the end of the 72 hour drying period, respectively while it was 66.2, 67.0, 67.9 and 73.6 brix in the Çalkarası grape.

3.2 Recovery of trans-resveratrol

Standard addition procedure was used for the recovery test. Thus, 0.2, 0.5, 1, 2 and 5 mg/L concentrations of trans-resveratrol standard were added to the five grape samples. In each addition level, three determinations were realized. The average percentage recoveries of trans-resveratrol in grapes were found to be 96.4%, 97.6%, 96.2%, 97.9% and 98.2% (average 97.3%).

3.3. Thermal Degradation kinetics of trans-resveratrol

Thermal stability of *trans*-resveratrol in seedless Sultana and Çalkarası were studied at 50, 55, 60 and 65 °C. As seen from Figure 1, thermal degradation of *trans*-resveratrol was fitted to first order reaction model. As the heating temperature and time increased, the degradation of *trans*-resveratrol increased.

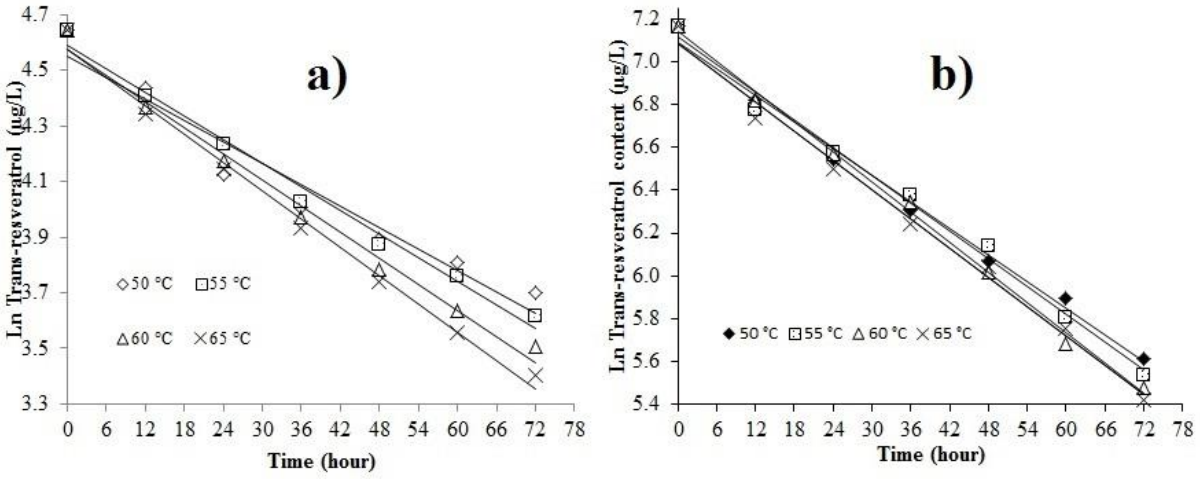


Figure 1. First order kinetics of *trans*-resveratrol degradation in a) seedless Sultana and b) Çal karasi grapes at four different temperatures.

Table 1 shows the degradation rate constants (k) and half-lives ($t_{1/2}$) for *trans*-resveratrol in seedless Sultana and Çalkarası, respectively. The linear inclination of the plot of $\ln(C/C_0)$ against *t* was used in order to estimate the degradation rate constant. The rate constants of *trans*-resveratrol increased with the increment of temperature in both varieties of grapes. This result suggests that degradations of *trans*-resveratrol are temperature-dependent. The results imply that *trans*-resveratrol was very unstable at 60°C as approved by higher *k* values resulting a fast degradation in Çalkarası grape, whereas degradation of *trans*-resveratrol in seedless Sultana was found at the highest level at 65°C.

As shown in Table 1, with the increment of temperature the half-life values ($t_{1/2}$) for *trans*-resveratrol decreased in both varieties of grapes. According to the $t_{1/2}$ values, *trans*-resveratrol degrades faster at higher temperatures. However, *trans*-resveratrol in Çalkarası grape showed the highest $t_{1/2}$ at 60°C when compared with seedless Sultana. According to Table 1, the highest thermal stabilities for *trans*-resveratrol in two grapes were found at 50°C.

Table 1. Effect of temperature on the reaction rate constant (k), activation energy (Ea), Q_{10} and half-life ($t_{1/2}$) values of *trans*-resveratrol in seedless Sultana and Çalkarası grapes.

Parameter	T (°C)	k 10^3 (1/hour)	$t_{1/2}$ (hour)	Q_{10} (50-65 °C)	E_a (kcal/mol)
Trans-Resveratrol (Seedless Sultana)	50	11.6	59.74	2.84	4.97
	55	13.7	50.58		
	60	15.2	45.59		
	65	16.4	42.25		
Trans-Resveratrol (Çalkarası)	50	20.7	33.47	1.64	1.63
	55	21.6	32.08		
	60	23.5	29.48		
	65	22.8	30.39		

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 2). Activation energies for *trans*-resveratrol degradation in seedless Sultana and Çalkarası grapes were different at temperature between 50-65°C. *Trans*-resveratrol showed the highest activation energy in seedless Sultana compared to Çalkarası. The results of Ea values in Table 1 and Table 2 suggest that *trans*-resveratrol in seedless Sultana is more stable to thermal degradation when compared with Çalkarası. The activation energies were calculated as 4.97 and 1.63 kcal/mol for the degradations of *trans*-resveratrol in seedless Sultana and Çalkarası, respectively.

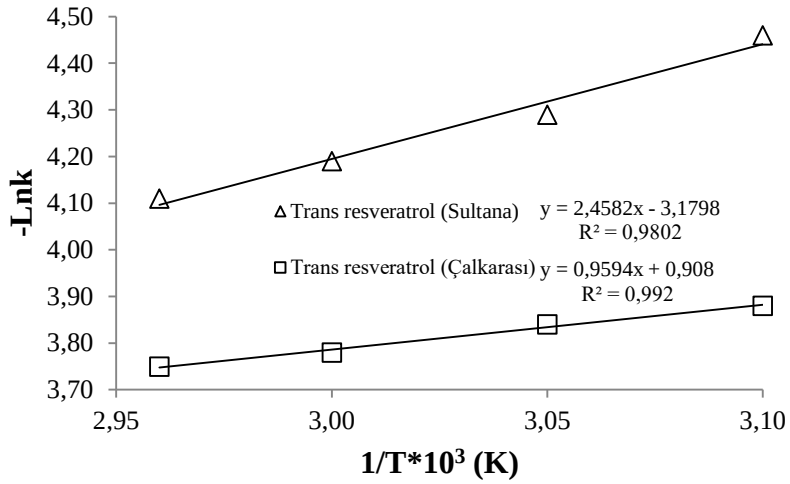


Figure 2. Arrhenius plots for degradation of *trans*-resveratrol in seedless Sultana and Çalkarası during tray drying.

Trans-resveratrol levels of samples, whose initial contents of *trans*-resveratrol were 107±0.07 and 1825±0.25 µg/L for seedless Sultana and Çalkarası, respectively decreased to 21±0.02 and 384±0.07 µg/L based on solar drying. *Trans*-resveratrol content of seedless Sultana and Çalkarası grapes at maturity stage were reported as 0.17 and 1.98 mg/kg, respectively (Otag & Kadakal 2015).

Degradation's percentage of *trans*-resveratrol was determined for seedless Sultana and Çalkarası as %80.37 and %78.95, respectively. Otag (2015) reported a 3.5-4 times loss in *trans*-resveratrol content of Çalkarası grapes during the solar and tray drying. As shown in Table 2, *trans*-resveratrol concentration of seedless Sultana and Çalkarası dried on tray dryer were found at 50°C and 65°C at the end of 72h as 43±0.01 and 32±0.01, 261±0.14 and 215±0.10, respectively. These results indicate that loss of *trans*-resveratrol in both grapes decreased dependent on increment of time.

Table 2. Percent decrement (%) of *trans*-resveratrol in seedless Sultana and Çalkarası grapes during solar and tray drying.

Type of Drying	Temperature	Time (hour)	Seedless Sultana (%)	Çalkarası (%)
Tray Drying	50 °C	12	27.9	29.3
		24	40.4	46.3
		36	47.1	57.7
		48	52.9	66.7
		60	56.7	71.5
		72	58.7	78.8
	55 °C	12	21.2	32.1
		24	33.7	44.3
		36	46.2	54.5
		48	53.8	64.2
		60	58.7	74.4
		72	63.0	80.3
	60 °C	12	24.0	29.3
		24	38.0	44.7
		36	49.0	56.1
		48	57.7	68.3
		60	63.5	77.2
		72	66.3	81.5
	65 °C	12	26.0	35.0
		24	39.4	48.8
		36	51.0	60.2
		48	59.6	67.5
		60	66.3	75.6
		72	69.2	82.5
Solar Drying		0	0	0
		252*	80.4	79.0

* Sun drying all day and night

4. CONCLUSION

Degradation of *trans*-resveratrol followed firs-order kinetics. As the temperature and tray drying time increased, degradation rate of examined compound increased. The best temperature for the less reduction of *trans*-resveratrol of seedless Sultana and Çalkarası grapes for long term tray drying were 50 °C. The dried grapes producing industry may benefit from the results of this study when controlling the amount of *trans*-resveratrol in production process. These kinetic parameters are important quality criteria for dried grapes. Therefore, knowing the degradation kinetic of *trans*-resveratrol is important.

To the best of our knowledge, there is no published study on the thermal degradation of *trans*-resveratrol of dried seedless Sultana and Çalkarası grapes.

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