

## **Modeling and Optimization of Ultrasound-Assisted Osmotic Dehydration with Finished Freeze Drying on Black Cherries – The Effect on Antioxidant Activities**

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**ABSTRACT:** In this study, response surface methodology was used to optimize ultrasound osmotic pretreatment with finished freeze drying of black cherries. Freeze drying is a separation process based on the sublimation phenomenon. This process has the following advantages as compared to the conventional drying process, maintenance of the structure, moisture removal at low temperature (reduced transport rates), improved stability of the product during storage, and minimization of degradation reactions. The effects of osmotic pretreatments (with or without ultrasound) in combination with freeze drying and hot-air drying methods on black cherries were investigated. Osmotic pretreatments and dehydration were carried out in sucrose solutions of 40, 50 and 60% and temperatures of 40, 50 and 60°C respectively. The influence of ultrasound at 65 and 130 KHz on antioxidant properties was also investigated. The total anthocyanins of dehydrated black cherries at higher temperatures as well as sucrose were decreased while the total phenol increased. Ultrasound caused a reduction in the antioxidant activity of the samples that were subjected to the increased amount of anthocyanins degradation. Freeze drying modified total anthocyanins, total phenolic compounds and the antioxidant activity in the final product.

**Keywords:** *Anthocyanin Content, Antioxidant Activity, Black Cherries, Osmotic Dehydration, Ultrasonic Pre-treatment.*

### **Introduction**

Drying is an ancient process used to preserve foods. The quality of dried products is dependent to on drying methods and conditions. Osmotic dehydration prior to drying has a protective effect on the structure of the dried material, making it more flexible. This reduces the loss of fresh fruit flavor, increases the sugar content and removes some acids, making osmotically-concentrated products acceptable (Ispir & Togrul, 2008). Mass transfer rates during osmotic dehydration depend on factors such as temperature, concentration of the osmotic medium, size and geometry of the sample,

sample to solution ratio, and intensity of agitation of the solution (Ispir & Togrul, 2008). The rate of mass transfer during osmotic dehydration is generally low. Application of ultrasound is to improve the mass transfer rates (Simal *et al.*, 1998) due to the effect of ultrasonic waves that cause a rapid series of alternative compressions and expansions, similar to a sponge being squeezed and released repeatedly (sponge effect). It generally precedes other processes such as freezing, freeze drying, vacuum drying or air drying (Chakraborty *et al.*, 2006). It is effective even at ambient temperatures, therefore heat damage to texture, colour and flavour can be minimized (Torreggiani, 1993). After this initial osmotic

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step, a complementary drying method is necessary to produce shelf-stable dehydrated products. Vacuum freeze-drying is the best method of water removal with final products of highest quality as compared to other methods of food drying (Ratti, 2001). Freeze-drying is based on dehydration by sublimation and deterioration and microbiological reactions are stopped that gives the final product the excellent quality.

*Prunus Serotina*, commonly called black cherry, wild black cherry, rum cherry, or mountain black cherry, is a woody plant species belonging to the genus *Prunus*. Black cherries are good sources of phenolic compounds and anthocyanins and also contain terpenes, phytochemicals that might help in the prevention of cancer. Interests in natural phenolic compounds in plants have been increasing due to their beneficial health effects namely the antioxidant activity. Many antioxidant compounds, naturally producing in plant sources have been identified as free radical or active oxygen scavengers (Silva *et al.*, 2007). The consequence of food processing and preservation procedure on the overall antioxidant activity of foods are generally the result of different events, hence the evaluation of processing factors influencing the antioxidant activity is imperative to increase or preserve their efficacy and bioavailability (Arabshahi *et al.*, 2007). Anthocyanins represent a unique subset of phenolic secondary metabolites found in plant tissues. They are one of many compound classes that fall under the flavonoid group, possessing a bi-phenolic structure (Barnes *et al.*, 2009). They are a group of plant pigments that are widely distributed in nature, among flowers, fruits and vegetables, and are responsible for their bright colors such as orange, red and blue. They play an important role in the plant physiology and are valuable for food industry as well as in human health (Patilet *al.*, 2009). Another significant property of

anthocyanins is their antioxidant activity that plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others (Corrales *et al.*, 2009).

The present study examined the effects of ultrasound-assisted osmotic dehydration and freeze finished drying on black cherries. Effects of concentration of sucrose (40, 50 and 60%), temperature (40, 50 and 60°C), frequency of ultrasound and freeze-drying on the antioxidant compounds of dried black cherries were also examined.

## Materials and Methods

### - Sample preparation

Black cherries were purchased from a local market. They were sorted based on their maturity and size. The cherries were washed and dried with absorbent paper. To increase permeability of the skin, they were dipped in NaOH (0.5 M) for 2 min. The average initial moisture content was  $80 \pm 1$  % (wet basis) which was measured gravimetrically using an oven at 105°C for 18h (Deng & Zhao, 2008). All measurements were carried out in triplicate order.

### - Ultrasonic assisted osmotic dehydration

Skin treated black cherries were weighed and placed into dehydrating vessel containing sugar solutions of varying concentrations (40-60% by weight). The vessel was placed into the ultrasonic bath and subjected to ultrasonic waves (0, 65 and 130 KHz) at a constant temperature (40-60°C) and sampling time (2-12h). The dehydrated samples from each group were drained and blotted with absorbent paper to remove excess solution. The average moisture and dry matter contents of the samples were measured according to AOAC method (AOAC, 1997). In this method, the ultrasonic bath is filled with a known amount of water (2L) and the water is

allowed to reach the equilibrium with room temperature. The ultrasound equipment is turned on and the water temperature is measured during operation every 30 minutes. In each experiment fresh sucrose syrup was used. All the experiments were done in triplicate order and the average values were taken for calculations.

#### - Freeze-drying

Osmotic dehydration was conducted when the moisture content of 30% (wet basis) was reached. Osmo dehydrated black cherries samples were then transferred to a freeze drier for the freeze-drying treatment. Freeze-drying treatment was conducted until the samples were completely dried.

#### - Hot-air drying

Osmo dehydrated black cherries were dried in a pilot plant hot-air drier (Tray dryer, Armfield, England). Drying was carried out at an air velocity of 1.5 m/s parallel to the drying surface of the samples at 70 °C. Hot-air drying was conducted until the samples were completely dried.

#### - Extraction of anthocyanins

The dried black cherries were homogenized using a commercial blender for 5 min. Five grams of samples were mixed with 20 mL of a solution containing ethanol and HCl 0.1 M (85:15) as a solvent and kept at 4°C for 12h with occasional vigorous shaking prior to centrifuging. The samples were centrifuged at 4000 g for 15 min at 10°C. The pellet was washed with 50 ml of ethanol containing 0.1M HCl (85:15) and centrifuged again under the same conditions (Stojanovic & Silva, 2007).

#### - Total anthocyanins

The total content of anthocyanins was determined by pH differential method (Stojanovic & Silva, 2007). Absorbance was measured by a UV/V is spectrophotometer (Cecil, CE 2502, England) in two levels of  $\lambda$

such as  $\lambda = 510$  nm and  $\lambda = 700$  nm in buffers of pH=1.0 and 4.5 using  $A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}$  with the molar extinction coefficient of cyanidin-3-galactoside (34700). Total anthocyanin was measured by equation 3 and the results were expressed as mg of cyaniding-3-galactoside equivalents per 100 g of the product and per 100 g of dry matter (Stojanovic & Silva, 2007).

$$\text{Total Anthocyanins } \left( \frac{\text{mg}}{\text{L}} \right) = \frac{(A \times \text{MW} \times \text{DF} \times 1000)}{\epsilon} \quad \text{Eq. 3}$$

Where MW is the molecular weight of cyanidin-3-galactoside (=502.5), A is the absorbance of the sample in spectrophotometer, DF is dilution factor and  $\epsilon$  is molar extinction coefficient for molar extinction coefficient (34700).

#### - Antioxidant activity

The antioxidant activity was measured by the EC<sub>50</sub> method using 0.1 ml of the appropriate extract mixed with 3.9ml of 25mg/l methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (Sigma, USA). The control sample was prepared with the same volume of methanol. Absorbance at 535nm was measured at different time intervals by spectrophotometer (CE 2502) until the reaction reached a steady state. The DPPH concentration in the reaction medium was calculated by linear regression using different concentrations of DPPH. The percentage of remaining DPPH (%DPPH<sub>Rem</sub>) at steady state was calculated as follows:

$$\% \text{DPPH}_{\text{Rem}} = \frac{[\text{DPPH}]_t}{[\text{DPPH}]_{t=0}} \quad \text{Eq. 4}$$

Where  $[\text{DPPH}]_{t=0}$  and  $[\text{DPPH}]_t$  are the initial concentrations of the DPPH and the DPPH concentration at a steady state, respectively. The percentage of remaining DPPH at a steady state was plotted against the sample concentration to obtain the EC<sub>50</sub>

value, that is the amount of sample necessary to decrease the initial DPPH concentration by 50%. EC<sub>50</sub> was expressed as mL of sample to g DPPH (Cam *et al.*, 2009).

- *Determination of total phenolic content*

After extraction of the phenolic compounds by the extraction methods described in previous sections, the concentration of total phenolic content was estimated by the Folin-Ciocalteu assay, according to the method presented by Lin and Tang (2007). Gallic acid was used as standard and results were expressed as mg of Gallic acid per 100g of dried sample.

- *Experimental Design and Statistical Analysis*

RSM with a central composite rotatable design (CCRD) was used to determine the optimum condition for osmotic dehydration and freeze drying. The experimental design and statistical analysis were performed using Minitab 16 software, English version (minitab Inc., State College, PA, USA). Concentration ( $x_1$ ), frequency ( $x_2$ ) and temperature ( $x_3$ ) in osmotic dehydration and freeze drying were selected as independent variables based on a literature survey and preliminary experiments (table1). Measures of responses were anthocyanins, total phenolic compounds and antioxidant activity of the samples. The complete design consisted of 20 combinations in osmotic dehydration and freeze drying (table 2).

Table 1. Independent variables and their coded and actual values used for optimization

Independent variable	Units	Symbol			
			-1	0	+1
Sugar Concentration	%	X <sub>1</sub>	40	50	60
Frequency	KHz	X <sub>2</sub>	0	65	130
Temperature	°C	X <sub>3</sub>	40	50	60

Table 2. Central composite rotatable design with the experimental values of the response variables

Run	Concentration(v/w) X <sub>1</sub>	Frequency(kHz) X <sub>2</sub>	Temperature(°C) X <sub>3</sub>	anthocyanin (mg/100g)	phenol (mg/100g)	EC50 (g)
1	50	65	50	1541.5	0.8651	0.0501
2	50	65	50	1557.4	0.8363	0.0503
3	40	0	40	4525.8	1.1732	0.0169
4	60	0	40	2268.2	1.0416	0.0220
5	40	130	60	3070.5	1.3149	0.0371
6	50	65	50	1325.7	0.9595	0.0502
7	60	130	40	1966.3	0.9249	0.0508
8	40	130	40	1825.1	0.9896	0.0415
9	50	65	50	1847.0	0.8795	0.0490
10	60	65	50	2549.2	1.2001	0.0593
11	40	65	50	3452.1	1.2433	0.0600
12	50	65	50	1557.4	0.8363	0.0533
13	40	0	60	2223.7	1.3611	0.0397
14	50	65	40	1899.7	0.8264	0.0331
15	60	0	60	1596.6	1.4071	0.0397
16	60	130	60	2811.2	1.444	0.0190
17	50	130	50	1358.4	0.8758	0.0508
18	50	0	50	1486.0	0.9020	0.0416
19	50	65	60	2203.8	1.3275	0.0343
20	50	65	50	1816.7	0.8683	0.05404

## Results and Discussion

### - Total anthocyanins, total phenolic compounds and $EC_{50}$

Total anthocyanins ( $Y_1$ ), total phenolic compounds ( $Y_2$ ) and  $EC_{50}$  ( $Y_3$ ) in dried black cherries are listed in table 2. These experimental data were used to calculate the coefficients of the second-order polynomial equations that are presented in table 3. For any terms in the model, a large regression coefficient and a small p-value would indicate a more significant effect on the respective response variables. ANOVA showed that the resultant second-order polynomial model adequately represented

the experimental data with the coefficient of multiple determinations for the response of total anthocyanins; total phenolic compounds  $EC_{50}$  ( $R^2$ ) of 0.91, 0.95 and 0.97, respectively.

Response surface analysis (RSA) of the data in Table 3 demonstrates that the relationship between the total anthocyanins, total phenolic compounds and  $EC_{50}$  and osmotic dehydration parameters is quadratic with good regression coefficient ( $R^2=0.91$ , 0.95 and 0.97). Eqs. (5, 6 and 7) show the relationship between total anthocyanins ( $Y_1$ ), total phenolic compounds ( $Y_2$ ),  $EC_{50}$  ( $Y_3$ ) and osmotic dehydration parameters ( $X_1$ ,  $X_2$  and  $X_3$ ).

$$\text{Anthocyanin}(Y_1)=45127.3-1280.48X_1+11.399X_1^2-0.10615X_2^2+0.532X_1X_2+0.9738X_2X_3 \quad \text{Eq.5}$$

$$\text{Phenol}(Y_2)=10.927-0.1262X_3+0.00263X_1^2+0.001183X_3^2 \quad \text{Eq.6}$$

$$EC_{50}(Y_3)=0.051+0.0039X_2+0.007X_1^2-0.006X_2^2-0.0187X_3^2-0.004X_1X_3-0.0095X_2X_3 \quad \text{Eq.7}$$

Table 3. The results of analysis of variance representing linear, quadratic and interaction terms of each variable and coefficient for the prediction model

source	df	anthocyanin		Total phenol		$EC_{50}$	
		Coef	P value	Coef	P value	Coef	P value
Model	9	45127.3	0.0003	10.927	< 0.0001	0.05172	< 0.0001
$X_1$	1	-1280.48	0.0035	-0.288	0.7668	-0.0004	0.6532
$X_2$	1	-63.139	0.3232	-0.002	0.1432	0.00393	0.0020
$X_3$	1	-327.032	0.5857	-0.126	< 0.0001	0.00055	0.5755
$X_1^2$	1	11.299	0.0002	0.0026	< 0.0001	0.00707	0.0029
$X_2^2$	1	-0.106	0.0453	-1.6E-05	0.1145	-0.0063	0.0056
$X_3^2$	1	1.810	0.3777	0.001	0.0149	-0.0188	< 0.0001
$X_1X_2$	1	0.532	0.0132	2.88E-05	0.4459	-0.0017	0.1329
$X_1X_3$	1	1.537	0.2109	0.0004	0.0778	-0.0040	0.0033
$X_2X_3$	1	0.973	0.0003	5.6E-05	0.1547	-0.0095	< 0.0001
Lack of fitness	5	0.0604		0.1059		0.1006	
$R^2$		0.914		0.9512		0.9701	
Adj- $R^2$		0.837		0.9072		0.94332	
CV		15.170		6.2825		7.0466	

$X_i$ , linear;  $X_i^2$ , quadratic and  $X_iX_j$ : interaction of variables (1: sugar concentration 2: frequency and 3: temperature).

Anthocyanins are a class of flavonoides that are soluble in water. They exist in epidermal and sub epidermal cells and dissolved in vacuoles or accumulated in vesicles called anthocyanoplasts therefore they can leak into the osmotic medium through the cuticle and skin ruptures of fruit. Layers of epidermal and subepidermal might be ruptured during osmosis due to the osmotic pressure gradient. The anthocyanin aren't stable and are affected by pH, light, heat, oxygen, metal ions like iron, copper and tin, sugar, electromagnetic waves and etc. The anthocyanin contents of berries are also influenced by the parameters of the osmotic process (sucrose concentrations, temperature), thus, the anthocyanin contents might be changed as the result of osmosis parameters changes.

This study examined the effects of sucrose concentrations, temperature variation and the application of ultrasound on the anthocyanin content and antioxidant activity of dried black cherries with freeze drying. Concentration of sugar had both linear and quadratic effects on the total anthocyanins ( $p < 0.001$ ).

The results showed that ultrasound pretreatment caused more leakage and loss of anthocyanins in black cherries since it creates cavitation phenomenon and this phenomenon caused the rupture of the surface on black cherries that allows the leakage of anthocyanins, but as long as ultrasound pretreatment led to much lower osmosis time, the anthocyanin contents of ultrasound samples was higher. (Carcelet *al.*, 2007; Fernandes *et al.*, 2007; Fernandes *et al.*, 2008)(Fig.1).

The total anthocyanins at different frequency were different. Although water loss increased with increase in frequency, the total anthocyanins at higher frequencies were greater because the sugar intake was

lower with increase in frequency (Fig.1). Increasing the sucrose concentration led to an increase in osmotic pressure and enhanced water loss. High water loss resulted in a greater loss of anthocyanins. The addition of sucrose to black cherries might also reduce the anthocyanins because of its destructive effect on them. This effect is because sugar addition might increase the pH of the solution and the samples and as the pH is increased, kinetic and thermodynamic competition occurs between the hydration reaction of the flavylium cation and the proton transfer reaction related to the hydroxyl groups and while the first reaction gives colourless carbinol bases, the latter reaction give rise to more violet quinoidal bases. When the percentage of anthocyanins in the colorless carbinol base is increased, the pigments became more susceptible to degradation by oxygen.

In this study, the temperature of the osmotic solution varied from 40 to 60°C. As the temperature is increased from 40 to 60°C, the anthocyanin content decreased because of the increased water loss and the destructive effect of temperature on the anthocyanins (Fig. 2).

Different osmotic concentration treatments induced loss of phenolics by migration into the osmotic solution. As shown in fig3 total phenol decreased with the increase of sugar concentration and increased with the increase of temperature. Introduction of ultrasound might accelerate the formation of free radicals and increases the level of polymerization of phenolics (Stojanovic & Silva, 2006). High frequency ultrasound, with its phenomenon of cavitation, may additionally rupture the surface of the black cherry, causing even more leakage and loss of phenolic compounds (Fig. 4).

Design-Expert® Software  
Factor Coding: Actual  
anthocyanin

4526  
1326

X1 = A: Concentration  
X2 = B: Frequency

Actual Factor  
C: Temperature = 50.00

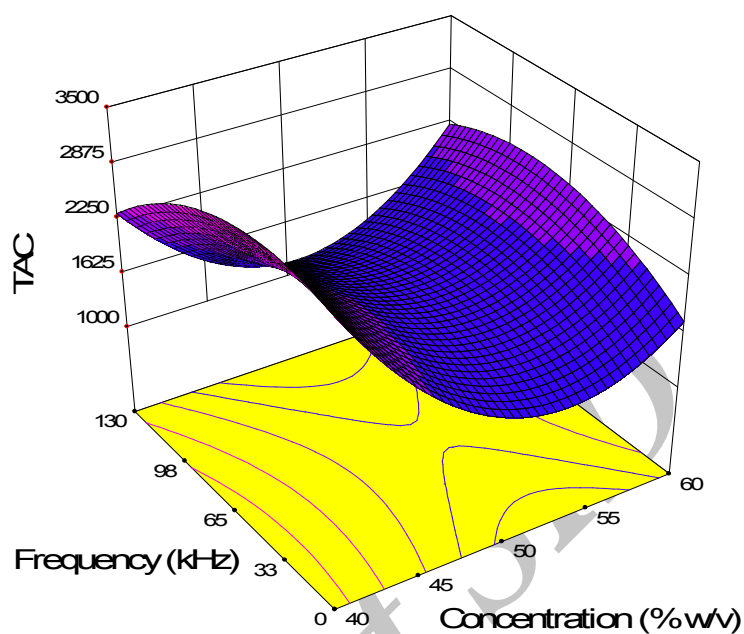


Fig. 1. Surface and contour plots for the effects of process concentration and frequency on anthocyanin.

Design-Expert® Software  
Factor Coding: Actual  
anthocyanin

4525.8  
1325.7

X1 = A: Concentration  
X2 = C: Temperature

Actual Factor  
B: Frequency = 65.00

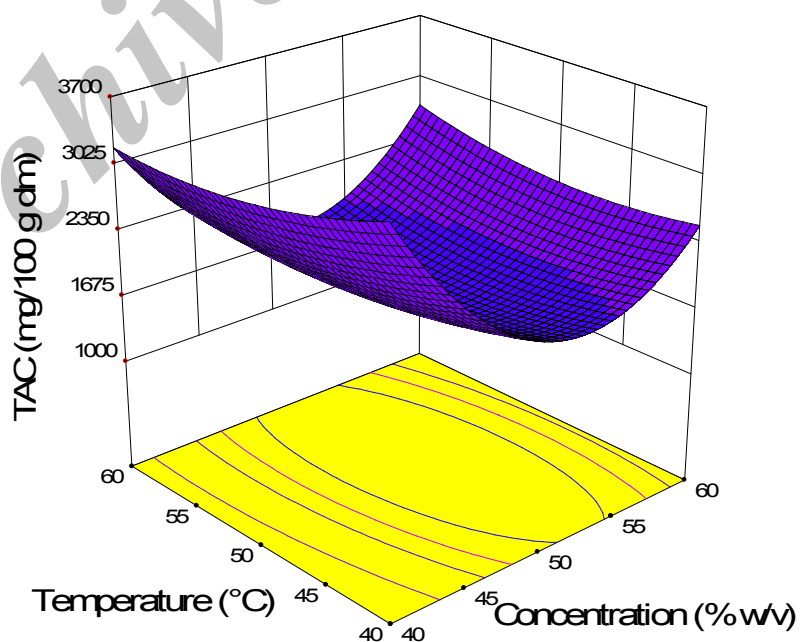


Fig. 2. Surface and contour plots for the effects of process concentration and temperature on anthocyanin.



Design-Expert® Software  
Factor Coding: Actual  
phenol



X1 = A: Concentration  
X2 = C: Temperature

Actual Factor  
B: Frequency = 65.00

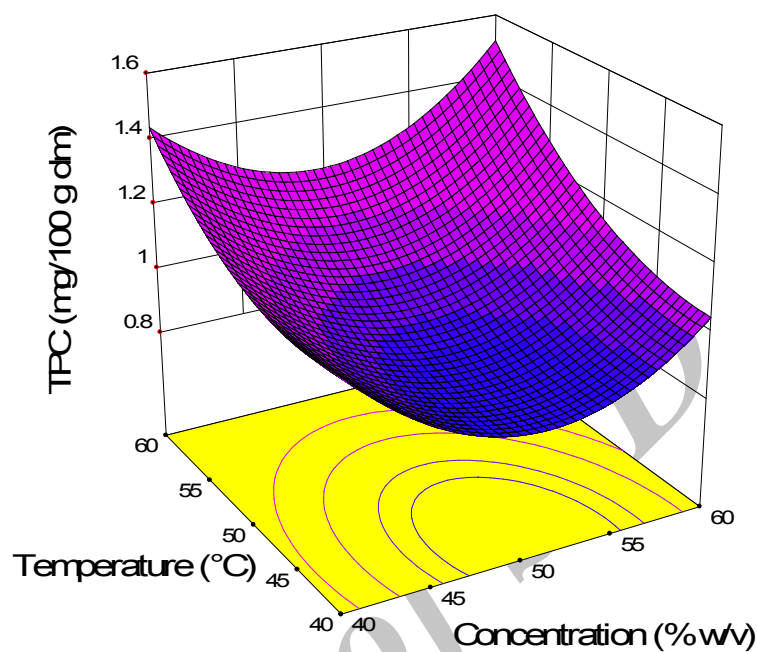


Fig. 3. Surface and contour plots for the effects of process concentration and temperature on total phenol.

Design-Expert® Software  
Factor Coding: Actual  
phenol



X1 = A: Concentration  
X2 = B: Frequency

Actual Factor  
C: Temperature = 50.00

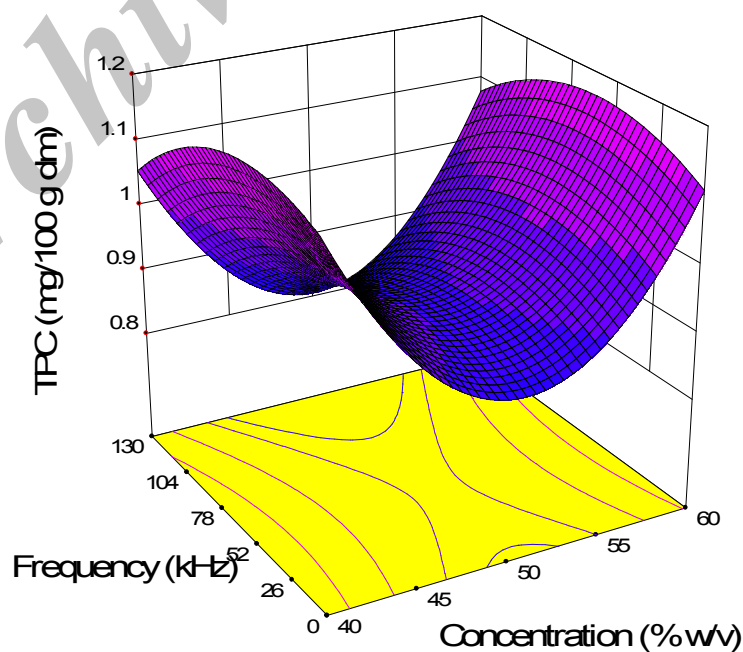


Fig. 4. Surface and contour plots for the effects of process concentration and frequency on total phenol.



#### - Antioxidant activity

Anthocyanins and phenolic compounds have strong antioxidant capacity and show different behaviors in response to temperature, oxygen and other processing factors. The reaction of antioxidants present in the black cherries extract with DPPH indicates quite a high activity.

The results showed that, although the anthocyanin and phenolic content of the samples were high in the ultrasonic pretreated samples, the antioxidant activity was low. This is because the ultrasound pretreatment enhanced the percent polymeric color that is an indication of anthocyanin degradation. As the percent polymeric color increased, antioxidant activity at 130 KHz decreased even more than at 35 kHz. While sucrose concentrations increased in the osmotic process, the changes in the antioxidant activity of the dried samples showed changes similar to that of the total anthocyanins and phenolic compound. Increasing the temperature decreased

antioxidant activity because of the decrease in total anthocyanins. The result showed that by increasing the temperature phenolic compounds are increased. Heat treatment affects the composition and distribution of phenolic compounds present in the extracts of citrus peels. Jeong et al. (2004) proposed that heat treatment might liberate phenolic compounds from an un-extractable form covalently bounded to the insoluble polymers (Figures 5 and 6).

#### - Optimization

Optimum conditions for black cherries with ultrasound osmotic dehydration pretreatment and freeze drying were determined to obtain maximum anthocyanin and phenolic compounds. The second-order polynomial models obtained in this study were utilized for each response in order to determine optimum conditions. The operating parameters were determined by considering economical, industrial and product quality constraints.

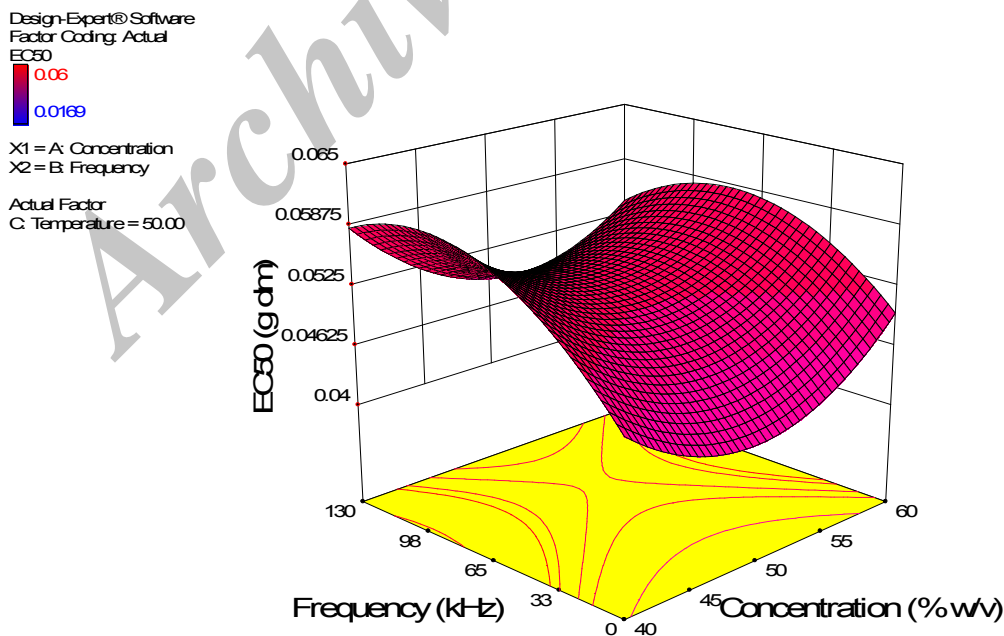


Fig 5. Surface and contour plots for the effects of process concentration and frequency on EC<sub>50</sub>.

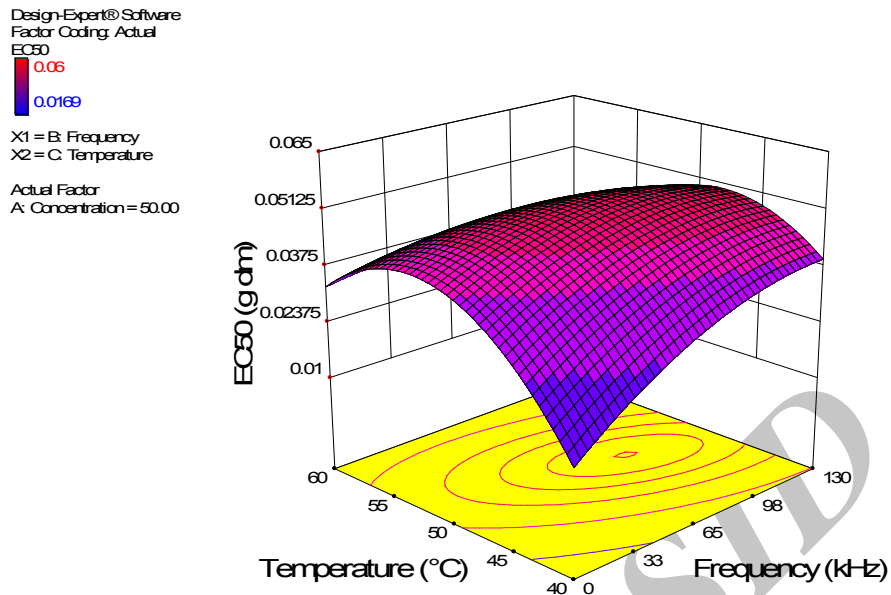


Fig. 6. Surface and contour plots for the effects of process temperature and frequency on  $EC_{50}$

In this study, the concentration of sugar, temperature and frequency were selected in the range of 40-60%, 40-60 °C and 65-130 KHz respectively. By applying the desirability function method, the optimum values were found to be 40% for concentration, 40°C for temperature and 35 KHz for frequency. The desirability value of the optimum extraction was 78%. The total anthocyanin, total phenolic compounds and  $EC_{50}$  were calculated as 4260 mg/100g, 1.17 mg/100g and 0.014g respectively after optimal dehydration conditions for drying of black cherries. The result of experiments and predicted values (means of three measurements) are presented in table 4 and indicates insignificant differences ( $p>0.05$ ) in the total anthocyanin, phenolic compound and  $EC_{50}$ , therefore corresponding models might be regarded suitable.

#### - Comparison of hot air and freeze drying as the finished drying methods

Hot air-drying is an ancient process used to preserve the foods in which the food to be dried is exposed to a continuous flow of hot stream of air where moisture is removed.

Unfortunately, the quality of a conventionally dried product is reduced as compared to the original product.

The finished drying method also affects the content of anthocyanins and phenolic compounds present in the dried samples. The total anthocyanins and phenolic compounds present in the freeze dried product were higher than the hot-air dried product because of the lower drying temperature (Andres *et al.*, 2004). The long drying time at high temperatures caused an increase in the production of furfural and 5-hydroxy methyl furfural that might degrade the pigment and enhances the negative influence of oxygen. The finished drying method also affected the antioxidant activity. Freeze drying increased the antioxidant activity because it increased the total anthocyanins and decreased the concentration of polymeric color (Figures 7 and 8).

#### Conclusion

The concentrations of sucrose, temperature, frequency and drying methods affected the total anthocyanins, total

phenolic compounds and the antioxidant activities in dried black cherries and as the sucrose concentration and temperature increased, the destruction of anthocyanins and leakage into the medium increased.

The samples having osmotic pretreatment combined with ultrasound had higher total anthocyanins but the antioxidant activities in these samples were lower than those treated with osmosis without ultrasound due to increased concentration of polymeric color.

The total anthocyanins and antioxidant activities of dried samples in the freeze drying method were higher than hot air method because this method was carried out at low temperature and therefore the destruction of anthocyanins decreased.

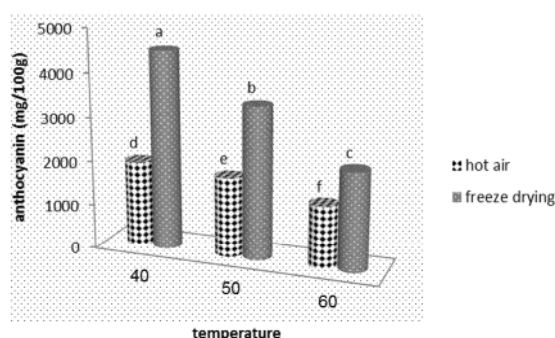


Fig. 7. The effects of changing method of finished drying (A) and temperature (B) on anthocyanin of dried black cherries. Different letters show significant statistical differences ( $p < 0.05$ )

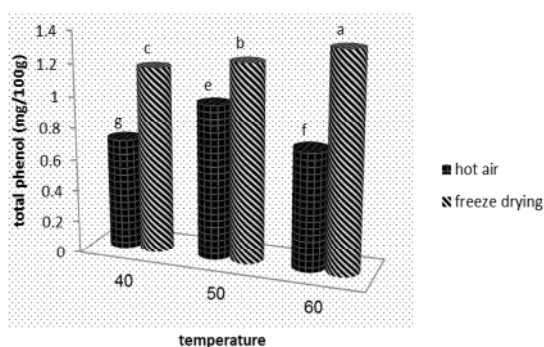


Fig. 8. The effects of changing method of finished drying (A) and temperature (B) on total phenol of dried black cherries. Different letters show significant statistical differences ( $p < 0.05$ )

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