Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi

Afyon Kocatepe University Journal of Science and Engineering

AKÜ FEMÜBİD **22** (2022) 015401 (154-164) AKU J. Sci. Eng. **22** (2022) 015401 (154-164)

DOI: 10.35414/akufemubid.1006595

Araştırma Makalesi / Research Article

Modeling the Effects of Physical Methods on Olive Bitterness Components

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Geliş Tarihi: 08.10.2021 Kabul Tarihi: 13.01.2022

Abstract

Olive fruit is rich in various micronutrients, especially phenolics and antioxidants, which are widely consumed as an important component of a healthy diet. In table olive production, chemical applications applied to reduce bitterness cause quality loss, salt accumulation and wastewater. Considering all these factors, within the scope of this study, the combined effects of sonication, freezing-thawing and drying processes, known as unique positive and successful applications, were investigated using RSM. For this purpose, total phenolic content (TPC), oleuropein and hydroxytyrosol content were examined while producing dry olives from ripe Edremit (Ayvalık) olives. It was found that raw Edremit olives had TPC at 16.28±0.58 mg gallic acid equivalents (GAE)/ g dry matter (DM). Oleuropein and hydroxytyrosol content at 15.39±1.70 mg/g DM and 0.544±0.06 mg/g DM, respectively. The quadratic model was found to be the most accurate in modeling and identifying TPC in experimental samples. According to ANOVA results, the most effective parameters on TPC were investigated as sonication time (A), drying temperature (C), interactive effect of sonication time and freezing temperature (AB), sonication time and drying temperature (AC) and the quadratic effect of sonication time (A^2). It was evaluated that, the combination of sonication, freezing and drying reduced the oleuropein content to 0.307-0.501 mg/g DM and hydroxytyrosol content to 0.135-0.202 mg/g DM regardless of the level of parameters ($p>0.05$). Consequently, it was proven that sonication, freezing- thawing and drying for desired level of TPC and oleuropein can be provided. For further studies, consumer expectation and sensory evaluation are required for targeted industrial applications.

Keywords
Physical Methods; Debittering; Table Olive; Salt Free

Fiziksel Yöntemlerin Zeytin Acılık Bileşenleri Üzerindeki Etkilerinin Modellenmesi

Öz

Zeytin meyvesi, sağlıklı beslenmenin önemli bir bileşeni olarak yaygın olarak tüketilen, fenolikler ve antioksidanlar başta olmak üzere, çeşitli mikro besinler açısından zengin bir hammaddedir. Sofralık zeytin üretiminde acılığı azaltmak için uygulanan kimyasal uygulamalar hem kalite kaybına hem de son üründe tuz birikmesine ve işleme sırasında atık su oluşumuna neden olmaktadır. Tüm bu faktörler göz önünde bulundurularak, bu çalışma kapsamında, benzersiz olumlu ve başarılı uygulamaları olduğu bilinen sonikasyon, donma-çözülme ve kurutma işlemlerinin birleşik etkileri, yüzey tepki yöntemi ilkesi kullanılarak araştırılmıştır. Bu amaçla olgun Edremit (Ayvalık) zeytinlerinden kuru zeytin üretilirken toplam fenolik içerik (TPC) ve daha spesifik olarak oleuropein ve hidroksitirosol içeriği incelenmiştir. Ham Edremit zeytinlerinin 16.28±0.58 mg gallik asit eşdeğeri (GAE)/g kuru madde (DM)'de TPC'ye sahip olduğu tespit edilmiştir. Oleuropein ve hidroksitirosol içeriği sırasıyla 15.39±1.70 mg/g DM ve 0.544±0.06 mg/g DM'de olarak bulunmuştur. TPC'yi modelleme ve tanımlamada kullanılan ikinci dereceden model, deneysel verilerde en uygun olarak bulunmuştur. ANOVA sonuçlarına göre,

Anahtar Kelimeler
Fiziksel Metotlar; Acılık Giderme; Sofralık Zeytin; Tuzsuz

sonikasyon süresi (A), kurutma sıcaklığı (C), sonikasyon süresi ve donma sıcaklığının etkileşimli etkisi (AB), sonikasyon süresi ve kurutma sıcaklığı (AC) ve sonikasyon süresinin ikinci dereceden (A2) etkisi TPC üzerinde en etkili parametreler olarak tespit edilmiştir. Sonikasyon, dondurma ve kurutma kombinasyonunun, parametre düzeyinden bağımsız olarak oleuropein içeriğini 0.307-0.501 mg/g DM'ye ve hidroksitirosol içeriğini 0.135-0.202 mg/g DM'ye düşürdüğü tespit edilmiştir ($p>0.05$). Sonuç olarak, sonikasyon uygulanarak, dondurularak-çözdürülerek ve kurutularak istenilen düzeyde TPC ve oleuropein sağlanabileceği kanıtlanmıştır. Sonraki çalışmalar kapsamında endüstriyel uygulanabilirliği tespit etmek için tüketici beklentisi ve duyusal değerlendirme çalışmaları gerekmektedir.

1. Introduction

Olive is a fruit that belongs to the Oleaceae family (Olea europea L.) and has a low sugar level and a high fat content when compared to other fruits in the species. Olives and olive products (table olives and olive oil) are essential elements of a healthy Mediterranean diet. A wide variety of bioactive compounds in the composition of olives are a remedy for many chronic and cardiovascular diseases. Many factors such as variety, maturation index, and method of removing bitterness affect the phenolic content of olives. Phenolics in olive fruit, leaves and seeds are generally grouped as a phenolic acid, phenolic alcohols, flavonoids and secoiridoids. Especially in fresh olive fruit, the dominant phenolic component is defined as "oleuropein" and since this compound is extremely bitter, consumption of the fruit is associated with the removal of this substance. Salt treatment and alkaline hydrolysis are among the most common techniques for this purpose. As a result of the hydrolysis of oleuropein, "hydroxytyrosol", the phenolic compound that is mainly responsible for the positive effect of olive on health, is formed (Charoenprasert and Mitchell, 2012, Habibi *et al*. 2015, 2016, Aydar *et al*. 2017b).

Although oleuropein which is synthesized by the olive fruit to protect itself, is destroyed with the degree of ripening, it is not possible to consume untreated olive as a table olive. Especially in the production of table olives, different production techniques such as Spanish style, Greek style, Californian style have been developed to reduce bitterness. In these methods, depending on the variety and the desired final product, olives are treated with chemicals (alkaline and/or salt) for 3-120 days and fermentation is applied for about 6 months. (Charoenprasert and Mitchell 2012, Habibi *et al*. 2015, 2016). Therefore, due to the fact that all these processes take time and increase the amount of salt in the final product, there is an increasing interest in accelerator and salt-free methods. In addition, the maximum amount of salt recommended for adults by the World Health Organization (WHO) and the British Food Standards Agency (FSA) has been determined as 5 g/day (~2000 mg Na/day). Studies conducted in different countries in Europe, North America and Asia show that the average salt consumption per capita is 12 g/day, well above the recommended amount. In addition to that although treatment with NaOH solution is the most frequently used method, as a result of this application, olives are subjected to more than one washing process in order to remove caustic (NaOH) from the olive, and as a result, both caustic and waste-water are formed in the factories. For this reason, it is extremely important for both public health and the environment that olive, which is an important food in daily nutrition, maintains the rich nutritional elements at the desired level, while being sensory acceptable and at the same time in a salt-free form (Anon.,2016). Innovative techniques that both reduce bitterness and preserve bioactive compounds in olives are gaining importance. For this purpose, techniques such as cold storage (Aydar *et al*. 2019), ultrasound application (Aydar, 2020) were used in the literature.

As a phyiscal treatment "Ultrasound (Supersonic)" literally means sound waves that vibrate at a higher frequency than the threshold of hearing. The range of 20 Hz – 20 kHz has been determined for the threshold of human hearing, and sounds in the range of 20 kHz – 1 MHz define the commercially studied ultrasound frequency range (T. Mason

1996, T. J. Mason & Lorimer 2002). In last decades, the use of ultrasound in hydrolysis, extraction and refining processes has gained great importance (Kardos and Luche 2001, Mason and Lorimer 2002, Fengs *et al*. 2010). In terms of extraction and partial hydrolysis, the main effects of ultrasound are defined as allowing the particles to absorb the solvent and accelerating the passage of the soluble compound from the particle to the medium. The mechanical effect of ultrasound can be listed as softening by hydrating, providing solvent penetration, increasing the transfer rate, tissue fragmentation and releasing bioactive compounds (Hromádková *et al*. 2008, Ebringerová and Hromádková 2010). When it is considered as the process of hydrolysis and extraction of oleuropein and ensuring the transition of phenolic substances to water in the washing process of olives, the use of ultrasound for bitterness removal can be considered as a good alternative. In some recent studies, it has been determined that olives, in which bitterness removal is applied by using ultrasound, minimize the quality loss and provide a time advantage compared to traditional methods. In addition, it has been determined that ultrasound has a positive effect on the bitterness reduction efficiency in the non-alkali processes (Aydar 2020, Habibi *et al*. 2015, 2016).

It is known that the freezing-thawing process also has some physical and biochemical effects on food substances (Heldman *et al*. 2006, Singh and Heldman 2013). The effect of refrigerated storing Gemlik variety olives at +4°C and freezing at -18°C, some physicochemical variations were observed on properties of olives as pH, acidity, total phenolic substance and oleuropein content. It was determined that there was a statistically significant change on the total amount of phenolic substances on the 7th day of freezing or cold storage of olives ($p<0.05$). It was observed that oleuropein degradation was found faster at frozen olives compared to refrigerated stored ones (Aydar *et al*. 2019). Freezing process can be an alternative to alkali application, by minimizing the amount of waste water without using any chemicals (Aydar *et al*. 2019, Aydar *et al*. 2018).

Olives can be consumed fresh, in the food industry it is be used as in dried form for pizzas, salads, sauces, and snacks. While the drying process extends the shelf life of the product, it is preferred in many countries due to the advantages it provides in preserving the original taste, aroma and nutritional elements. From this point of view, dry olive production has a significant meaning in terms of new product development and olive preservation (Aydar *et al*. 2016, Yılmaz and Aydar 2016, İçier *et al*. 2014, Mahdhaoui *et al*. 2013). During the drying process, there will be a decrease in the bitterness components depending on the temperature. For this reason, the bitterness removal process in the study also includes the effect of the drying system. Considering that phenolic substances are sensitive to heat, heat application is a preferable method, but in this case, hydroxytyrosol, may be lost as well as bitterness components. (Charoenprasert & Mitchell, 2012). It has been stated that heat treatment applied to olives at 30-50^{o}C for up to 72 hours in order to reduce bitterness has a significant effect on bitterness, especially above 40^{o}C, and 24-hour treatment found to be sufficient (García *et al*. 2001).

The goal of this study was to maximize the positive effects of each debittering application while limiting the negative effects, and to see if subsequent processes have positive symbiotic effects on removal of bitterness. Within the context of the pattern given in the method section, first ultrasonic washing, then quick freezing and frozen storage, and finally drying operations were used as the application sequence. Considering that the target product is dried olives, it serves the purpose that the steps are in this order.

2. Material and Methods

In this study, pitted green Edremit variety olives were obtained from the local company. Olives used in this study were harvested by hand in Akhisar/Manisa region during the early harvest period of September 2020-2021 and stored at 4^{o}C until the analysis and process. The maturity index (MI) of pitted olives (3.67) was determined by the

method described by Aydar *et al*. 2017a. It was reported that, fungal infections and physiological damage are prevented in olives stored at 5°C for up to 8 weeks, however, maturation index of the olives increases, and the bitterness components are destroyed (Yousfi *et al*. 2008). Therefore, maximum 1 week of storage was applied to olive samples. Standards and chemicals such as potassium iodide, chloroform, sodium carbonate, starch solution, acetic acid, potassium persulfate, sodium thiosulfate, Trolox solution, Folin-Ciocalteu reagent, in analytical grades were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.1. *Physicochemical analysis:*

Moisture content of the olive puree was determined by dry oven method at 105˚C (AOAC 971.28,1998). The total phenolic content analysis was carried out with the Folin-Ciocalteu method. Briefly, Folin-Ciocalteu reagent was added to homogenized product and reading was carried out at 765nm wavelength in the spectrophotometer (Danahaliloğlu *et al*. 2018)

The phenolic fraction extracted was analyzed by high-performance liquid chromatography (HPLC). HPLC used in this study was an Agilent Technologies 1200 series system equipped with an automatic injector, a column (C18, 4.6x100mm inner diameter, 2.7µm particle size) and a diode array UV detector (Agilent Technologies, Palo Alto, CA). The flow rate, the injection volume and column temperature was 0.45 mL/min, 2 µL and 25 °C, respectively. The mobile phase contained A:0.1% formic acid and B:methanol. Standards of polyphenolic compounds of oleuropein and hydroxytyrosol were purchased from Merck and Sigma–Aldrich, respectively.

2.2. *Treated dry olive production:*

In ultrasound assisted washing, 400 grams of pitted olives was sonicated in 4 L distilled water (Alex, 32±5 Hz/Turkey) in an ultrasonic bath for 10, 20, 30 minutes, and the temperature data was noted using design were represented in Table 1. The effect of parameters as sonication time, freezing temperature and drying temperature were K-type thermocouples. Washed pitted olives were dried with the help of paper and frozen at different freezing temperatures (-20°C, -30°C, -40°C) in the individual quick freezer (IQF) than stored at -18°C until drying step which is less than a week. Before the drying process, frozen products were left at room temperature for 10 minutes to thaw. The olive drying process was carried out in an industrial tray dryer (Eksis Machine/Turkey). Preliminary experiments were made to determine the drying conditions and the temperatures were determined as 50°C, 60°C and 70°C and the air velocity was 1 m/s. The system was stopped and trays were weighed every 30 minutes and the process was stopped when the humidity level reached 25±2% (dry weight), which is the most preferred level in the preliminary studies (Aydar *et al*. 2016) ,and the final products were stored at 4°C in vacuum packages until they were analyzed (Fig. 1).

2.3. *Experimental design and evaluation:*

Design Expert 11 package program was used for experimental design and data analysis. For the most appropriate definition in a 3-parameter design, the suitability of the quadratic (2nd Degree) model defined in equation 1 was investigated. “Y” is the predicted value; “a_0” is constant; The values “a_o”, a_{ii}”and “a_{ij}” are the regression constants of the intersection, linear, quadratic and interaction terms.

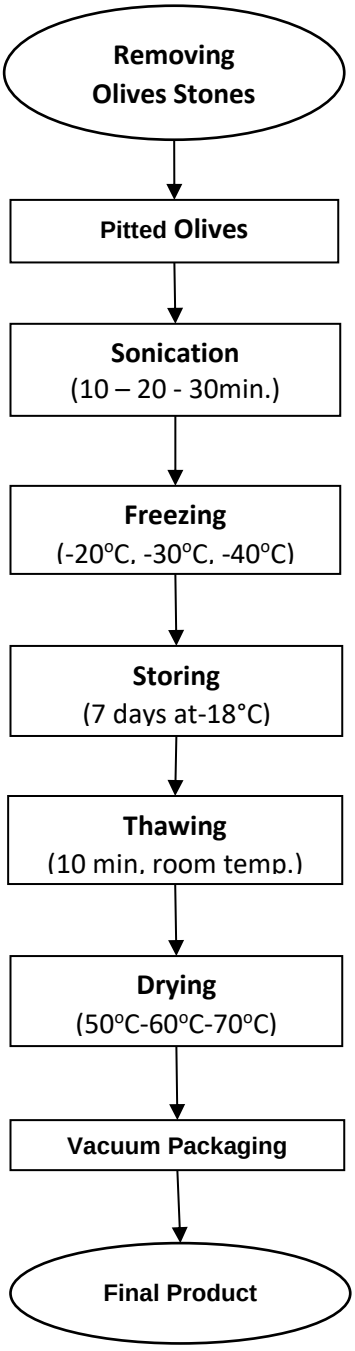

Figure 1. Flow diagram of unsalted dried olive processing

"X_i" and "X_j" indicates the levels of the independent variables. The values of the coefficients are determined with the analysis of variance (ANOVA) tables.

$$Y = a_0 + \sum_{i=1}^{3}(a_i X_i) + \sum_{i=1}^{3}(a_{ii} X_i^2) + \sum_{i=1}^{3} \sum_{j=1}^{3}(a_{ij} X_i X_j) \quad (1)$$

The independent variable levels in equation 2 are encoded value of the independent variable, x_i, the current value of the independent variable, x_0, the value of the independent variable at the center point, Δxi, the amount of change of the independent variable.

$$x_i = \frac{X_i - X_0}{\Delta X_i} \pi r^2 \quad (2)$$

The significance of the factors was determined by calculating the Fisher's ratio of variance (F-value) calculated for the confidence intervals. With this ratio calculation, the acceptability of the regression coefficients was determined. Surface and contour graphs are obtained with fit polynomial equations; The relationships between the levels of the factors and the responses were visualized (Prakash Maran & Manikandan, 2012). Analysis of variance was performed to determine the matching ratio of experimental data and predicted values. The regression coefficient of the model (R2), the number (MSE), root of mean square error (RMSE) and of corrected regressions (adj-R2), the number of estimation regressions (pred-R2), the coefficient of variation (CV), random error variance estimator residual error sum of squares (PRESS) values were calculated and interpreted in the results section. (Montgomery, 2000, Myers vd. 2009). The descriptive-diagnostic tests of the selected model were performed, and the independence of the residual errors from each other and their normal distribution were examined and interpreted. Whether the "Leverage" value is less than 1 and the number of middle values are also checked. (Mei *et al*. 2009, Prakash Maran and Manikandan 2012, Milić *et al*. 2013, Elksibi *et al*. 2014).

3. Results and Discussion

TPC in raw olive samples was found 16.28±0.58 mg GAE/ g DM. This value is compatible with previous studies which were 12.92 and 18.53 mg GA/ g DM in Edremit variety and Gemlik variety in raw form respectively (Tokuşoğlu *et al*. 2010). In a study on a green olive *(Carolea cv.*) which was harvested in south Italy the TPC was found 13.64±0.64 mg GAE/g DM. (Piscopo *et al*. 2014). In raw form there were slight differences observable within different variety of olive due to region, climate and maturity. TPC in mg GAE/ g DM of the final products of experimental

Table 1. Experimental results

Run order	Stn. order	Sonication Time (min.)	Freezing Temperature (°C)	Drying Temperature (°C)	TPC (mg GAE/ g DM)	Oleuropein (mg/g DM)	Hydroxytyrosol (mg/g DM)
14	1	10	-30	50	7,60	0,12	2,39
10	2	20	-20	50	7,21	0,23	8,73
5	3	20	-40	50	4,71	0,04	0,58
9	4	30	-30	50	9,60	0,05	1,47
12	5	10	-20	60	8,67	0,40	20,56
15	6	10	-40	60	5,73	1,50	0,08
4	7	30	-40	60	9,62	0,96	0,08
8	8	30	-20	60	6,82	10,14	0,18
7	9	10	-30	70	6,40	0,61	10,40
17	10	20	-20	70	3,78	0,81	32,71
11	11	20	-40	70	6,53	0,18	5,94
6	12	30	-30	70	7,25	0,51	12,98
1	13	20	-30	60	6,32	8,41	0,19
2	14	20	-30	60	5,89	5,00	0,21
3	15	20	-30	60	6,19	4,18	0,25
16	16	20	-30	60	5,86	8,60	0,22
13	17	20	-30	60	6,26	3,02	0,13

investigated in ANOVA table (Table 2). Quadratic model was found the best to predict experimental results compared to other alternatives by representing highest R^2 (>0.95), and insignificant "lack of fit" ($p<0.05$).

Table 2. ANOVA table of BBD

Source	Sum of Squares	F-value	p-value
Model	36,91	38,14	< 0.0001
A-Sonication time	2,98	27,74	0,0012
B-Freezing temp.	0,0016	0,0146	0,9071
C-Drying temp.	3,34	31,05	0,0008
AB	8,25	76,70	< 0.0001
AC	0,3299	3,07	0,1233
BC	6,88	64,03	< 0.0001
A^2	14,86	138,17	< 0.0001
B^2	0,3162	2,94	0,1301
C^2	0,3064	2,85	0,1352
Residual	0,7527		
Lack of Fit	0,5729	4,25	0,0980
error	0,1797		
Total	37,66		
Mean vs Total	770,49		
Linear vs Mean	6,32	0,8743	0,4795
2FI vs Linear	15,46	3,25	0,0685
Quadratic vs 2FI	**15,12**	**46,89**	**< 0.0001**
Cubic vs Quadratic	0,5729	4,25	0,0980

Table 3 Regression coefficients of the model

Parameter	Value
Constant	+6,10
A-Sonication time	+0,6106
B-Freezing temp.	-0,0140
C-Drying temp.	-0,6460
AB	-1,44
AC	-0,2872
BC	-1,31
A^2	+1,88
B^2	-0,2741
C^2	-0,2698

Therefore, developed mathematical model was found reliable to explain responses under the identified levels of parameters. For more safe prediction, diagnostic analyses were carried out as given in Figure 2 to see whether the predicted values could show angle of 45° with the experimental results at given run. Also, residuals were found in acceptable distance and deviations were lower than 1 as given in Leverage test (Fig 2).

C.V. %	4,87
R^2	0,9800
Adj R^2	0,9543
Pred R^2	0,7491
Adeq Precision	24,5760

After considering the quadratic model was safe and reliable, regression coefficients of the coded values were calculated as given in Table 2 and response graphs were evaluated (Fig 3).

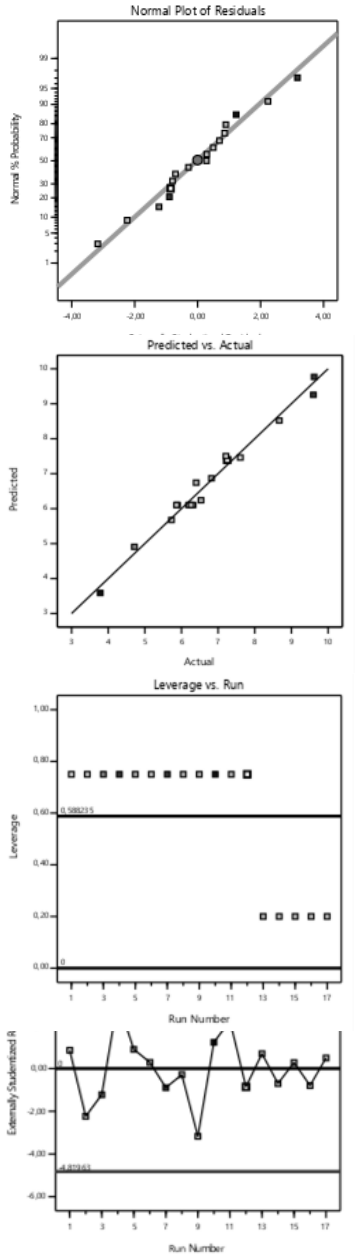

Figure 2. Diagnostic graphs

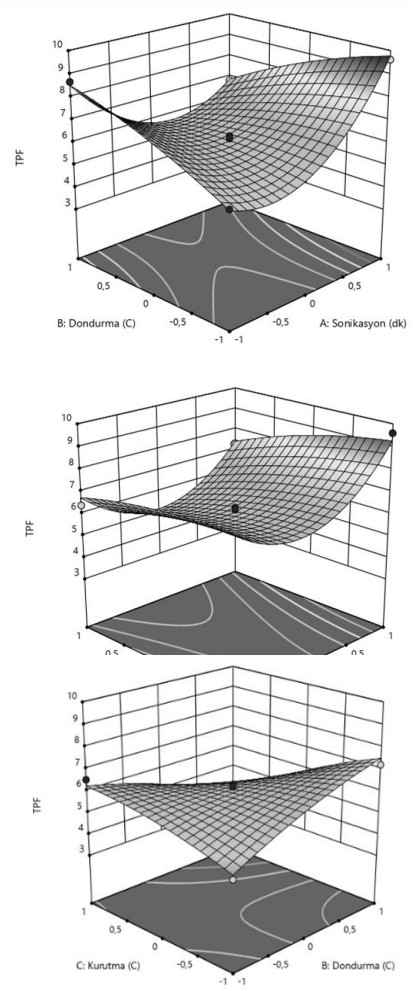

Figure 3. Surface graphs

From the Table 2, the significant parameters on total phenolic content were the linear effect of sonication time (A) and drying temperature (C), interactive effect of sonication time and freezing temperature (AB), sonication time and drying temperature (AC) and the quadratic effect of sonication time (A^2) ($p<0.05$). The final equation of yield in terms of coded significant factors ($p<0.05$) was found as:

$$TPC = 6.10 + 0.6106A - 0.6460C - 1.44AB - 0.2872AC + 1.88A^2 \quad (3)$$

More specifically the major phenolic compounds in olive as oleuropein and hydroxytyrosol were investigated both in raw and processed olives. In this study, oleuropein and hydroxytyrosol content of raw olives were calculated as 15.39±1.70 mg/g DM and 0.544±0.06 mg/g DM respectively. In a olives variety Nocellera

del Belice these values were 11.63±0.10 mg/g DM and 0.537±0.002 mg/g DM; green California type 16.50 mg/g DM and 0.570mg/g DM (Ambra *et al*. 2017). Slight difference between varieties were observable having similar reason with TPC as mentioned above. Bitterness content oleuropein is need to be degrade due to some ripening and processing methods including alkali, enzymatic, heating and non-thermal methods. As a non-thermal application high hydrostatic pressure was applied to raw Ayvalık and Gemlik variety olive samples and oleuropein content was reduced to 0.5185-1.910 mg/g DM while hydroxytyrosol content was increased up to two-fold (Tokuşoğlu *et al*. 2010). This phenomenon was expressed by oleuropein degradation and hydroxytyrosol formation. In a study on California type olive processing, final olives have oleuropein at 0.100 mg/g DM and hydroxytyrosol at 3.95 mg/g DM which is 7-fold increase in hydroxytyrosol content. Olives used in this study, were stored under anaerobic conditions in 8% NaCl brine for 4 months, before use. The process was continued for 3 days with 0.5 M NaOH. During the alkali application, water addition and aeration processes were carried out. When the pH reaches 8.00, (5 g/l) ferrous gluconate solution was added, and air was bubbled for another 24 h. Then analyzes were carried out.

On the other hand, due to process severity and variety hydroxytyrosol content was increased and then decreased due to degradation or removal. In a Spanish style table olive processing the hydroxytyrosol content of Nocellera del Belice decreased with processing steps. In this study, olives were kept in aqueous NaOH solution (2.9 baume degree) at room temperature for 12 hours. Then, olives were washed three times with water. Afterwards, stored in polyethylene barrels containing 7% salt. Seven samplings were performed during the process: time 0 (raw olives), after 12 h of NaOH processing, after 4 and 8 days and 1, 2 and 7 months from the beginning of the fermentation process. (Ambra *et al*. 2017, Marsilio *et al*. 2001).

In our study the combination of sonication, freezing and drying dramatically effected the oleuropein and hydroxytyrosol content at various level. For the center point, which was 20 min sonication, freezing at -30°C and drying at 60°C the oleuropein and the hydroxytyrosol content were found as 5.84±2.26 mg/g DM and 0.2±0.04 mg/g DM respectively. However, considering all experiments the oleuropein content varies between 0.12 to 10.14 mg/g DM and the hydroxytyrosol content was found between 0.13 to 32.71 mg/g DM. This situation hinders to evaluate the effect of parameters on given phenolic substances. In terms of response surface modelling, none of the model fit to explain the effects of each parameter on oleuropein and hydroxytyrosol level. Negative value at predicted R^2 was achieved for oleuropein prediction while only linear model found suitable for hydroxytyrosol at predicted R^2 at 0.17 with very important lack of fit value ($p<0.0001$). Therefore, only experimental data were collected to identify the level of each phenolics, further investigations were carried out using the TPC values of experiments. On the other hand, considering the results of previous researchers, the final product developed in this study has an appropriate level of oleuropein, but the content of hydroxytrosol was increased by formation or reduced due to removal or degradation in some cases caused by sonication, freeze-thaw, and heat treatment. For instance, ultrasound application is a technology applied in extraction processes as mentioned before, and there are many studies show that the transition of the active compound to the solvent medium is accelerated depending on the ultrasound duration. In a study on phenolic removal of olive fruit, the sonication was found more effective than hot water application by removing almost all oleuropein (16.563 ± 1.720 mg/g DM) while only half of the oleuropein removed (9.208 ± 0.317 mg/g DM) in hot water treatment. However, hydroxtyrosol content of the final product was found similar for all treatments. In addition, probe-type high-energy ultrasound represented higher efficiency in phenolic removal compared to bath application (Jerman *et al*. 2010, Deng *et al.* 2017). In another study of phenolic

extraction from olive leaves, several drying temperatures and sonication were applied. It was figured out that sonication accelerates phenolic transition from matrix to the solution (Khemakhem *et al*. 2017). Similarly, in obtaining oleuropein from the olive leaf matrix, sonication was found to be more effective than traditional extraction (Ahmad-Qasem *et al*. 2013).

Freezing and thawing is a complex phenomenon in where physicochemical changes observed. In this study Gemlik variety olives were stored at +4^{o}C cold storage and -18^{o}C frozen storage for 7 to 35 days. Consequently, TPC and oleuropein decrease was observed for each storage while freeze-thaw caused higher decrease (Aydar, 2020). In another study frozen storage at -18^{o}C and -25 ^{o}C for 90 days were applied to Gemlik variety olives. 27.35% and 31.74% reduction in oleuropein was calculated for -18^{o}C and -25^{o}C storage respectively (Kayguluoğlu, 2018).

Heat treatment has dominant effect on TPC. In a study Gemlik and Ayvalık variety olive leaves were dried at various conditions by using various methods as conventional hot air, microwave, and infrared resulting in oleuropein content at 0.092-0.142 mg/g DM (Kara, 2013). Another study on olive leaves represented that both sonication and heat accelerate TPC and oleuropein removal from the leaf matrix (Khemakhem *et al.* 2017). In a Carolea cv variety olives were dried at 50^{o}C and 70^{o}C. TPC was calculated as 0.013 mg/g DM in raw olives, and it was reduced to 4.54 mg GAE/ g DM for 50^{o}C and 11.38 mg GAE/g DM for 70^{o}C drying. Additionally, oleuropein content was reduced from 4.161 mg/g to 0.002 mg/g DM and 0.005 mg/g DM respectively at 50^{o}C and 70^{o}C drying (Piscopo *et al*. 2014).

4. Conclusion

Physical debittering methods such as sonication, freezing-thawing and drying were used to overcome salt accumulation problem on the final product. Combination effect of parameters on the phenolic content was investigated using response surface methodology. TPC in raw olive samples was found 16.28±0.58 mg GAE/ g DM. Major phenolic components as oleuropein and hydroxytyrosol content of raw olives were calculated as 15.39±1.70 mg/g DM and 0.544±0.06 mg/g DM respectively. Quadratic model was found the best to predict TPC content in experimentally obtained samples. The linear effects of sonication time, drying temperature, interactive effect of freezing and drying temperatures and quadratic effect of sonication were defined as the most significant parameters on TPC. The combination of sonication, freezing and drying reduced the oleuropein content to 0.307-0.501 mg/g DM and hydroxytyrosol content to 0.135-0.202 mg/g DM regardless of the level of parameters ($p>0.05$). As a result, it has been demonstrated that sonication, freezing-thawing, and drying methods can achieve the desired level of TPC and oleuropein in olives. In future studies, sensory evaluations will be required in order to apply the findings to the industry and meet consumer expectations.

Acknowledgement

This article has been prepared by using the data produced This work was supported by TÜBİTAK Project (Project code: 120O310) We would like to thank Aydar Zeytincilik A.Ş. for supplying the olives used in the study.

5. References

Ahmad-Qasem, M. H., Cánovas, J., Barrajón-Catalán, E., Micol, V., Cárcel, J. A., García-Pérez, J. V. 2013. Kinetic and compositional study of phenolic extraction from olive leaves (var. Serrana) by using power ultrasound. *Innovative Food Science and Emerging Technologies*, ***17***, 120–129.

Ambra, R., Natella, F., Bello, C., Lucchetti, S., Forte, V., Pastore, G. 2017. Phenolics fate in table olives (Olea europaea L. cv. Nocellara del Belice) debittered using the Spanish and Castelvetrano methods. *Food Research International*, ***100***, 369–376.

Aydar, A. Y., Bağdatlıoğlu, N., Köseoğlu O. 2017a. Determination the Effect of Ultrasound on Olive Oil Extraction and Optimization of Ultrasound-Assisted Extraction of Extra Virgin Olive Oil by Response Surface Methodology (RSM). *Grasas y Aceites. International Journal of Fats and Oils*, **63**(2) e189.

Aydar, A. Y., Öncü Öner, T., Üçok, E. F. 2017b. Effects of Hydroxytyrosol on Human Health. *EC Nutrition*, ***11***(October), 147–157.

Aydar, A. Y. 2020. Quality Parameters and Drying Kinetics of Ultrasound Pretreated Fermented Black Table Olives. *Latin American Applied Research*, ***50***(4), 271–276.

Aydar, A. Y., Özçelik, M., Pazarlıoğlu, B. 2019.. The Effects of Storage of Gemlik Olives at Different Temperatures on Oleuropein Degradation, 3rd International Conference on Agriculture, Food, Veterinary and Pharmacy Sciences (ICAFOP) 16-18 April 2019,Trabzon, Turkey

Aydar A. Y., Yılmaz T., Öncü Öner T. 2018. Methods for Reducing Bitterness of Table Olives, II. International University Industry Cooperation,R&D and Innovation Congress Manisa Celal Bayar University, 14-15 November 2018, Manisa, Turkey

Aydar, A. Y., Yılmaz, T., Tepekule, H., Çapan, G., Obuz, E. 2016. 4th International Food RD Brokerage Event. 24 Mayıs 2016. İzmir,Turkey.

Charoenprasert, S., Mitchell, A. 2012. Factors influencing phenolic compounds in table olives (Olea europaea). *Journal of Agricultural and Food Chemistry*, ***60***(29), 7081–7095.

Danahaliloğlu, H., Tekeli, Y., Göycıncık, S., Yıldırım, F. 2018. Hatay'da Farklı Bölgelerde Yetiştirilen Zeytin Yapraklarının Antioksidan Özelliklerinin Belirlenmesi. *Karadeniz Fen Bilimleri Dergisi*, ***8***(1), 77–86.

Ebringerová, A., Hromádková, Z. 2010. An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides. *Central European Journal of Chemistry*, ***8***(2), 243–257.

Elksibi, I., Haddar, W., Ticha, M. Ben, Mhenni, M. F. 2014. Development and optimisation of a non conventional extraction process of natural dye from olive solid waste using response surface methodology (RSM). *Food Chemistry*, ***161***, 345–352.

Feng, H., Barbosa-Canovas, G. V., Weiss, J. 2010. Ultrasound Technologies for Food and Bioprocessing. Springer, 1-643.

García, J. M., Yousfi, K., Mateos, R., Olmo, M., Cert, A. 2001. Reduction of oil bitterness by heating of olive (Olea europaea) fruits. *Journal of Agricultural and Food Chemistry*, ***49***(9), 4231–4235.

Habibi, M., Golmakani, M. T., Farahnaky, A., Mesbahi, G., Majzoobi, M. 2016. NaOH-free debittering of table olives using power ultrasound. *Food Chemistry*, ***192,*** 775–781.

Habibi, M., Golmakani, M. T., Mesbahi, G., Majzoobi, M., Farahnaky, A. 2015. Ultrasound-accelerated debittering of olive fruits. *Innovative Food Science and Emerging Technologies*, 105–115.

Heldman, Dennis R., Daryl B. Lund, Christina Sabliov, E. 2006. *Handbook of food engineering*. CRC Press, 427-471.

Hromádková, Z., Kost'álová, Z., Ebringerová, A. 2008. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. *Ultrasonics Sonochemistry*, ***15***(6), 1062–1068.

İçier, F., Baysal, T., Taştan, Ö., Özkan, G.2014. Microwave Drying Of Black Olive Slices : Effects On Total Phenolic Contents And Colour. *Gıda*, ***39****(6)*, 323–330.

Kara, S. 2013. Farklı Kurutma Yöntemlerinin Zeytin Yaprağındaki Fenolik Madde Dağılımına Ve Antioksidan Kapasitesine Etkisinin Araştırılması*, (*Yüksek Lisans Tezi), T.C. Balıkesir Üniversitesi Fen Bilimleri Enstitüsü, Balıkesir, 57.

Kardos, N., Luche, J. 2001. Sonochemistry of carbohydrate compounds. *Carbohyrdate Research*, ***332****(2)*, 115–131.

Kayguluoğlu, A. 2018. Sofralık Siyah Zeytin Kalitesi Üzerine Acılık Giderme İşlemlerinin Etkisi, (Doktora Tezi), T.C. Bursa Uludağ Üniversitesi Fen Bilimleri Enstitüsü, Bursa, 163.

Khemakhem, I., Ahmad-Qasem, M. H., Catalán, E. B., Micol, V., García-Pérez, J. V., Ayadi, M. A., Bouaziz, M. 2017. Kinetic improvement of olive leaves' bioactive compounds extraction by using power ultrasound in a wide temperature range. *Ultrasonics Sonochemistry*, **34**, 466–473.

Mahdhaoui, B. Mechlouch ,R.F., Mahjoubi, A., Karim Zahafi, K., Ben Brahim, A.2013. Mathematical model on thin layer drying of olive fruit (Olea europeae L. *Journal of Agricultural Technology* , **9(5)**, 1097-1110.

Marsilio, V., Campestre, C., Lanza, B. 2001. Phenolic compounds change during California-style ripe olive processing. *Food Chemistry*, ***74*(**1), 55–60.

Mason, T. 1996. The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, ***3***(3), S253–S260.

Mason, T. J., Lorimer, J. P. 2002. Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing, Wiley, 225-266.

Mei, L., Zhen-Chang, W., Hao-Jie, D., Li, C., Qing-Gang, X., Jing, L. 2009. Response surface optimization of polysaccharides extraction from Liriope roots and its modulatory effect on Sjogren syndrome. *International Journal of Biological Macromolecules*, ***45***(3), 284–288.

Milić, P. S., Rajković, K. M., Stamenković, O. S., Veljković, V. B. 2013. Kinetic modeling and optimization of maceration and ultrasound-extraction of resinoid from the aerial parts of white lady's bedstraw (Galium mollugo L.). *Ultrasonics Sonochemistry*, ***20***(1), 525–534.

Montgomery, D. C. 2000. Design and Analysis of Experiments, 5th Edition. John Wiley & Sons, New York, NY, 427-511.

Myers, R. H., Montgomery, D. C., Anderson-Cook, C. 2009. Response Surface Methodology: Process and Product Optimization Using Designed Experiments . *Wiley Series in Probability and Statistics*, *June 2015*, 631-693.

Piscopo, A., De Bruno, A., Zappia, A., Poiana, M. 2014. Antioxidant activity of dried green olives (Carolea cv.). *LWT - Food Science and Technology*, ***58***(1), 49–54.

Prakash Maran, J., Manikandan, S. 2012. Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (Opuntia ficus-indica) fruit. *Dyes and Pigments*, ***95***, 465–472.

Singh, R. P., Heldman, D. 2013. Introduction to Food Engineering 5th Edition.Elsevier, 549-561.

Tokuşoğlu, Ö., Alpas, H., Bozoğlu, F. 2010. High hydrostatic pressure effects on mold flora, citrinin mycotoxin, hydroxytyrosol, oleuropein phenolics and antioxidant activity of black table olives. *Innovative Food Science and Emerging Technologies*, **11**(2), 250–258.

Yousfi, K., Cayuela, J. a, García, J. M. 2008. Reduction of virgin olive oil bitterness by fruit cold storage. *Journal of Agricultural and Food Chemistry*, ***56***(21), 10085–10091.