

## **VALORIZATION OF OLIVE-OIL INDUSTRY SOLID WASTE: STATISTICAL OPTIMIZATION OF ALKALINE EXTRACTION CONDITIONS FOR LIGHT-COLOURED HEMICELLOUSES**

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Received/ Geliş: 14.11.2016; Accepted / Kabul: 06.03.2017; Online baskı / Published online: 23.03.2017

Sutay Kocabaş, D, Yurtdaş, E, Demir, AN, (2017). Valorization of olive-oil industry solid waste: Statistical optimization of alkaline extraction conditions for light-coloured hemicelluloses. *GIDA* (2017) 42 (3): 329-338 doi: 10.15237/gida.GD16100

### **Abstract**

Olive solid waste (OSW), the by-product of olive oil production process, is a lignocellulosic material with very low economic value. The hemicellulose fraction of OSW has a potential to be used as a raw material for several industrial applications. The main objective of the present work was to statistically develop an alkaline hemicellulose extraction procedure to achieve the highest possible crude hemicellulose extraction yield from OSW with concurrent efforts to lighten the colour of black hemicelluloses. Box-Behnken Design based response surface methodology was employed for optimization. The optimum hemicellulose extraction conditions were found as 19.3% (w/v) alkaline (KOH) concentration, 1.3 mm particle size, 25.3 h extraction time, 1% (w/v) MgSO<sub>4</sub> concentration, 6.4% (v/v) H<sub>2</sub>O<sub>2</sub> concentration and 60°C temperature. The maximum experimental hemicelluloses yield at this condition was 35.6±0.5% which is in agreement with the predicted yield value of 31.2%. As a result, the extraction yield has been increased from 21.7% to 35.6% (w/w). The colour values (L\*,a\*,b\*) of hemicelluloses extracted at optimum conditions were predicted as 31.69±0.28, -2.90±0.04 and 5.49±0.04, sequentially. The findings of this study demonstrated the route for isolation of light-coloured OSW hemicelluloses that have potential for conversion into industrially important value-added products such as biodegradable plastics for food packaging applications and xylooligosaccharides.

**Keywords:** Olive solid waste, pretreatment, hemicellulose, extraction, optimization, response surface methodology, waste valorization

## **ZEYTİNYAĞI ENDÜSTRİSİ KATI ATIĞININ DEĞERLENDİRİLMESİ: AÇIK RENKLİ HEMİSELÜLOZLAR İÇİN ALKALİ ÖZÜTLEME KOŞULLARININ İSTATİSTİKSEL OPTİMİZASYONU**

### **Öz**

Zeytinyağı üretim sürecinin yan-ürünü olan zeytin katı atığı (ZKA), çok düşük ekonomik değere sahip bir lignoselülozik materyaldir. ZKA'nın hemiselüloz fraksiyonu, birçok endüstriyel uygulama için hammadde olarak kullanım potansiyeline sahiptir. Bu çalışmanın temel amacı, ZKA'dan mümkün olan en yüksek ham hemiselüloz özütlemeye verimine ulaşmak için bir alkali özütleme tekniğinin istatistiksel olarak geliştirilmesi ve bunun yanında siyah hemiselülozların renginin açılmasıdır. Optimizasyon için Box-Behnken Tasarımı temelli tepki yüzey metodolojisi kullanılmıştır. Optimum hemiselüloz özütleme koşulları %19,3 (a/h) alkali (KOH) konsantrasyonu, 1,3 mm partikül büyüklüğü, 25,3 saat özütleme süresi, %1 (a/h) MgSO<sub>4</sub> konsantrasyonu, %6,4 (h/h) H<sub>2</sub>O<sub>2</sub> konsantrasyonu ve 60°C sıcaklık olarak tespit edilmiştir. Bu koşullardaki en yüksek deneysel hemiselüloz verimi %35,6±0,5 olup, teorik değer olan %31,2 ile uyumludur. Sonuç olarak, özütleme verimi %21,7'den %35,6'ya (a/a) çıkarılmıştır. Optimum koşullarda özütlenen hemiselülozların renk değerleri (L\*,a\*,b\*) sırasıyla 31,69±0,28, -2,90±0,04 ve 5,49±0,04 olarak bulunmuştur. Çalışmanın sonuçları, gıda paketeleme uygulamaları için biyobozunur plastikler ve ksilooligosakkaritler gibi endüstriyel öneme sahip katma değerli ürünlere dönüştürülme potansiyeline sahip açık renkli ZKA hemiselülozlarının izole edilmesi için yol göstermektedir.

**Anahtar kelimeler:** Zeytin katı atığı, ön işlem, hemiselüloz, özütleme, optimizasyon, tepki yüzey metodolojisi, atık değerlendirme

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## INTRODUCTION

Plant biomass components such as lignocellulosic residues have attracted more attention within the last two decades due to the rapid decrease in the amounts of fossil resources (1). Lately, many efforts have been employed for the conversion of biomass and other waste residues into new value-added products, within valorization approach (2). Biomass is a sustainable and renewable resource for the production of biofuels, materials and green chemicals and it is a putative source for carbon-based products with a remarkable reduction of CO<sub>2</sub> release into the atmosphere (3, 4). Lignocellulosic biomass is made up of three types of biopolymers: cellulose (45-55%), hemicellulose (25-35%), lignin (20-30%) and hemicelluloses are the most abundant biopolymers after cellulose. Despite of the huge production rate, hemicelluloses are still wasted during bio-based processes. On the contrary, hemicelluloses have many attractive properties including biodegradability, biocompatibility and bioactivity. Due to these excellent features, hemicelluloses are used in many industrial areas such as food, medicine, energy, chemicals and polymeric materials production, hence they are in the focus of growing scientific interest (5). In the last decade, hemicelluloses have received increasing attention for the production of biodegradable films and coatings because the bulk of engineered plastic materials used today are made from petroleum-based synthetic polymers which generate many problems due to their non-renewable nature and ultimate disposal (6, 7). A superior property of hemicellulose (xylan) based films is their low oxygen permeability, which makes them attractive in certain food packaging applications (7). Xylan based films also possess good intrinsic barrier properties against polar migrants such as aromas (8). In addition, hemicelluloses are raw materials for oligosaccharides which act as potential prebiotics (9). Encapsulation applications of hemicelluloses are reported for food and pharmaceutical use (10, 11). Therefore, extraction of hemicelluloses from different low-cost and non-food lignocellulosic feedstocks makes them attractive for further studies. The composition of hemicelluloses varies between type and source of feed stocks, their origin and growth stage. In lignocellulosic structure,

hemicellulose is bound to cellulose and lignin and detailed isolation procedures are necessary to separate these components (12). Different extraction techniques have been performed and generally heat treatment is combined with alkali, acid or hydrogen peroxide (13). Biomass pretreatment is the most expensive step in bioprocesses representing about 20% of the total cost which limits the conversion of biomass into products (14, 15). Therefore, optimization of pretreatment methods has a great economical importance in terms of industrial applications. Alkali extraction is a chemical pretreatment technique which can be performed at room temperature and times ranging from seconds to days. Alkali extraction causes less sugar degradation than acid pretreatment and it is more effective on agricultural residues than on wood materials (16). A mild alkaline peroxide process has shown to provide a successful delignification with minimal loss of lighter-in-colour hemicelluloses (17).

The olive (*Olea europaea*) is an evergreen tree cultivated for the production of table olives and oil. Globally, Mediterranean region is the dominating olive production area, where olive oil production has been started more than 7000 years ago. Olive cultivation has also spread globally during past two decades due to the health benefits assigned to olive oil. In year 2013, global olive production exceeded 20 million tonnes (1, 18, 19). The liquid and solid non-food residues of these milling processes have great negative environmental impact on microbial population of soil, aquatic ecosystems and air (20). Depending on the oil extraction technology used, every 100 kg of olives generates 35-45 kg of olive solid waste (OSW). To valorize this waste, there has been increasing attention on the re-use and recycling potential of OSW for the production of value-added products (21). Thus, the aim of the study was to maximize the amount of the extracted crude hemicellulose from OSW by a two-phase statistical optimization process, with concurrent efforts to lighten its colour. Overall, six extraction process parameters were studied. In the first phase; alkali (KOH) concentration, OSW particle size, extraction time, and in the second phase; MgSO<sub>4</sub> concentration, H<sub>2</sub>O<sub>2</sub> concentration and temperature were optimized. Specifically, the effect of H<sub>2</sub>O<sub>2</sub> on the colour of

hemicelluloses was investigated. While several studies dealing with OSW are available, to the best of our knowledge, there is no study in literature which reports optimization of light-coloured hemicellulose extraction from OSW.

**MATERIALS AND METHODS**

**Materials**

OSW of olives grown in Aegean region of Turkey was obtained from Uşaklı Pirina (Aydın, Turkey). Without any treatment, OSW was ground to a particle size according to the experimental design and was further used in optimization studies.

**Experimental design**

The conditions for hemicellulose extraction from OSW were optimized in two phases by using response surface methodology (RSM) based on Box-Behnken Design (BBD). In the first optimization phase, three independent variables were tested: alkali (KOH) concentration ( $X_1$ ), particle size ( $X_2$ ) and time ( $X_3$ ). In the second phase, the results of the first phase were used as the starting point and further optimization was performed by testing another three independent variables:  $MgSO_4$  concentration ( $X_4$ ),  $H_2O_2$  concentration ( $X_5$ ) and temperature ( $X_6$ ). The range and levels of the independent variables are given in Table 1. For each optimization phase, 15 experimental runs with different combinations of the three selected variables were performed in two replicates with three center points according to the BBD matrix. Minitab® Release 17 Statistical Software (USA) was used for the analysis of the data. A full quadratic model was adopted to predict

the optimum points according to Equation 1;

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \quad \text{Equation 1}$$

where Y is the predicted response (hemicellulose extraction yield, %),  $b_0$  intercept (constant),  $b_i$  linear coefficients,  $b_{ii}$  squared coefficients,  $b_{ij}$  interaction coefficients and X is the coded level of the independent variable.

**Extraction of hemicellulose fraction**

The hemicellulose extraction method was based on the alkaline extraction procedure described by Zilliox and Debeire (1998) and Bahcegul et al. (2014), with some modifications (22,7). OSW was ground to a particle size according to the experimental design and 10 g OSW was washed with 200 ml of distilled water. The suspension was filtered and OWP was transferred into 80 ml of alkaline (KOH) solution at given concentration and the extraction was set for proper hours at room temperature. At the end of pretreatment, the alkaline insoluble residue (cellulose fraction) was collected by centrifugation. Alkaline soluble hemicellulose was precipitated by the addition of 250 ml of acetic acid:ethanol (1:10, v/v) solution. The mixture was left to precipitate for 10 minutes and hemicellulose fraction was collected by centrifugation. Hemicellulose particles were dried at room temperature (45±5% humidity) to constant weight.

The optimum conditions predicted in the first optimization phase were the starting points of the second optimization phase. The primary aim of this extraction phase was to maximize the hemicellulose yield and the secondary purpose was to obtain hemicelluloses which are lighter in colour. To obtain light-colored hemicelluloses

Table 1. The levels of independent variables of BBD

Optimization phase-1	Independent variable code	Independent variable name	Level		
			-1	0	+1
	$X_1$	Alkali (KOH) concentration (%)	10	20	30
	$X_2$	Particle size (mm)	1	2	3
	$X_3$	Time (h)	6	24	42
Optimization phase-2	Independent variable code	Independent variable name	Level		
			-1	0	+1
	$X_4$	$MgSO_4$ concentration (%)	0	0.5	1
	$X_5$	$H_2O_2$ concentration (%)	0	4	8
	$X_6$	Temperature (°C)	20	40	60

H<sub>2</sub>O<sub>2</sub> was used, which delignifies plant materials with subsequent colour change (17). Prior to alkaline peroxide extraction, 10 g of grounded and sieved OSW was weighted and washed with chelate agent, 200 ml ethylenediamine tetraacetic acid (EDTA) solution (1%, w/v), for 1 hour. The use of EDTA is necessary before H<sub>2</sub>O<sub>2</sub> treatment because EDTA removes the metal ions from the solution. The metal ions were reported to decompose H<sub>2</sub>O<sub>2</sub> and reduce delignification process performance (13). After EDTA treatment, OSW was washed thoroughly with distilled water until the pH of filtrate became 7.0. OSW was filtered to remove the excess water and was transferred into alkaline solution which was an aqueous solution of KOH, MgSO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. It is known that chelation not only removes heavy metals, but also alkali earth metals which act as stabilizer of H<sub>2</sub>O<sub>2</sub> during extraction. Therefore, MgSO<sub>4</sub> was added to increase the magnesium concentration, thus to stabilize H<sub>2</sub>O<sub>2</sub>. Proper volume of H<sub>2</sub>O<sub>2</sub> was added into the KOH solution (19.3%, w/v) and a calculated amount of MgSO<sub>4</sub> was mixed and volume of the solution was adjusted to 80 ml. Extraction process was carried out in a thermostat-controlled water bath at given temperature. Likewise the prior extractions, liquid part was removed by centrifugation, hemicellulose was precipitated by the addition of 250 ml of acetic acid:ethanol (1:10, v/v) solution. The obtained hemicellulose was dried at room temperature (45±5% humidity) and the yield was calculated after hemicellulose reached the constant weight.

In both of the optimizations phases, hemicelluloses yield values (% w/w) were calculated according to Equation 2. The statistical analysis was performed for independent variables and the optimum conditions for the maximum hemicellulose extraction yields were determined. The optimum conditions were verified experimentally with four replicates.

$$\text{Hemicelluloses yield (\%)} = \frac{\text{Dry weight of extracted hemicelluloses (g)}}{\text{Dry weight of olive solid waste (g)}} \times 100$$

Equation 2

### Colour Measurement

The colour of hemicelluloses was determined by using 30% (w/v) aqueous solutions of hemicellulosic fractions at room temperature.

The colour measurement was performed by using a colorimeter (Hunterlab ColorFlex CFLX-45, USA) which was calibrated with standard black and white calibration plates. The Hunterlab color scale (L\*,a\*,b\*) was used for analysis. The hemicellulose samples were analyzed in four replicates and the results were given as mean ± standard deviation.

## RESULTS AND DISCUSSION

### Optimization of hemicellulose extraction conditions (Phase-1)

In the first optimization phase, a series of experiments were conducted in order to determine the influence of three extraction parameters (alkali concentration, particle size, time) on the yield of hemicellulose extraction. The experimental design and hemicellulose yield results are given in Table 2. The observed hemicellulose yields were in the range of 7.8 to 22.4%. The best fit was observed by using full quadratic model and the results of regression analysis are given in Table 3. Equation 3 was attained with a high coefficient of determination, *R*<sup>2</sup> value of 0.904, indicating that 90.4% of the variability in the response could be explained by the full quadratic model equation. *T* and *P* (probability) values are used to predict the significance of each coefficient and interactions between the variables. As an accepted rule, the smaller the *P*-value (generally less than 0.05) and larger the *T* value, the more significant the corresponding coefficient (23). Among the independent variables, OSW particle size (*X*<sub>2</sub>) showed a significant effect on hemicellulose extraction yield. The negative sign of particle size coefficient demonstrates an effect to decrease the extraction yield, whereas positive coefficient of time indicates an effect to increase the yield. The square term of time variable has a *P*-value below 0.05 and it showed a significant negative effect on the response. Analysis of variance (ANOVA) was performed to check the statistical significance of the full quadratic model. A low value of *P* (<0.05) and a high value of *F* (5.20) indicated that the model is statistically significant (data not shown).

$$\text{Hemicellulose yield (\%)} = 21.70 - 0.01 X_1 - 3.89 X_2 + 1.40 X_3 - 3.46 X_1 X_2 - 2.96 X_2 X_3 - 7.24 X_3 X_1 + 0.78 X_1 X_2 + 0.05 X_1 X_3 + 0.70 X_2 X_3 \quad \text{Equation 3}$$

Table 2. Experimental design of two-phase optimization strategy and hemicellulose extraction yields (% w/w) on dry weight basis

	Run	KOH concentration ( $X_1$ )	Particle size ( $X_2$ )	Time ( $X_3$ )	Experimental hemicellulose yield (%)	Predicted hemicellulose yield (%)	Residual
Optimization phase-1	1	1	1	0	9.70	12.15	-2.45
	2	1	-1	0	20.40	18.37	2.03
	3	-1	1	0	8.60	10.62	-2.02
	4	-1	-1	0	22.40	19.95	2.45
	5	1	0	1	12.70	12.44	0.26
	6	-1	0	-1	9.40	9.66	-0.26
	7	1	0	-1	9.70	9.54	0.16
	8	-1	0	1	12.20	12.36	-0.16
	9	0	1	1	11.90	9.71	2.19
	10	0	-1	-1	12.50	14.69	-2.19
	11	0	1	-1	7.80	5.51	2.29
	12	0	-1	1	13.80	16.09	-2.29
	13	0	0	0	21.50	21.70	-0.20
	14	0	0	0	21.30	21.70	-0.40
	15	0	0	0	22.30	21.70	0.60
	Run	MgSO <sub>4</sub> concentration ( $X_4$ )	H <sub>2</sub> O <sub>2</sub> concentration ( $X_5$ )	Temperature ( $X_6$ )	Experimental hemicellulose yield (%)	Predicted hemicellulose yield (%)	Residual
Optimization phase-2	1	-1	0	-1	12.62	12.332	0.288
	2	0	0	0	21.94	20.420	1.520
	3	0	1	1	23.92	23.989	-0.069
	4	0	0	0	19.46	20.420	-0.960
	5	1	0	1	30.16	30.447	-0.287
	6	-1	1	0	15.60	16.416	-0.816
	7	-1	-1	0	12.67	13.026	-0.356
	8	1	1	0	23.06	22.704	0.356
	9	1	0	-1	11.18	12.065	-0.885
	10	-1	0	1	16.55	15.665	0.885
	11	0	-1	1	17.75	18.279	-0.529
	12	0	-1	-1	10.78	10.711	0.069
	13	0	0	0	19.86	20.420	-0.560
	14	0	1	-1	10.37	9.841	0.529
	15	1	-1	0	22.07	21.254	0.816

The contour plots of three independent variables studied in the first optimization phase against hemicellulose extraction yield (response) were used to further understand the results of the statistical analysis (Figure 1-a). The contour plots revealed that the response surface had a maximum point which is the optimum extraction condition for the first phase of optimization. The optimum point for maximum crude hemicellulose extraction yield was found as; -0.0707 for  $X_1$  (19.3% KOH concentration), -0.6566 for  $X_2$  (1.3 mm particle size) and 0.0707 for  $X_3$  (25.3 h time), which was verified experimentally with four replicates. The maximum hemicellulose yield obtained at optimized conditions was found as 25.0%. This result was in agreement with the yield of 23.0%, predicted by the model.

**Optimization of hemicellulose extraction conditions (Phase-2)**

The experimental design and hemicellulose yield results of second phase are given in Table 2. Levels of hemicellulose yield ranging from 10.37 to 30.16% were observed. The best fit was observed by using full quadratic model and the results of regression analysis are given in Table 3. The coefficient of determination ( $R^2$ ) was found as 0.984 which indicates a high correlation (98.4%) between the experimentally observed and predicted values, thus the significance of the model. Regardless of the significance of coefficients, a second order polynomial equation (Equation 4) was predicted. According to the low  $P$ -values (<0.05) and positive coefficients, all linear variables showed a significant positive impact on hemicelluloses extraction yield. The highest

Table 3. Estimated regression coefficients for hemicellulose extraction

	Term	Coefficient	T	p
Optimization phase-1	Constant	21.70	13.13	0.000
	KOH concentration	-0.01	-0.01	0.991
	Particle size	-3.89	-3.84	0.012
	Time	1.40	1.38	0.225
	KOH concentration * KOH concentration	-3.46	-2.32	0.068
	Particle size * Particle size	-2.96	-1.99	0.103
	Time * Time	-7.24	-4.86	0.005
	KOH concentration * Particle size	0.78	0.54	0.612
	KOH concentration * Time	0.05	0.03	0.973
	Particle size * Time	0.70	0.49	0.646
S = 2.318 R-Sq = 90.4% R-Sq(adj) = 73.0%				
	Term	Coefficient	T	p
Optimization phase-2	Constant	20.420	29.01	0.000
	MgSO <sub>4</sub> concentration	3.629	8.42	0.000
	H <sub>2</sub> O <sub>2</sub> concentration	1.210	2.81	0.038
	Temperature	5.429	12.59	0.000
	MgSO <sub>4</sub> concentration * MgSO <sub>4</sub> concentration	-0.074	-0.12	0.912
	H <sub>2</sub> O <sub>2</sub> concentration * H <sub>2</sub> O <sub>2</sub> concentration	-1.996	-3.15	0.025
	Temperature * Temperature	-2.719	-4.28	0.008
	MgSO <sub>4</sub> concentration * H <sub>2</sub> O <sub>2</sub> concentration	-0.485	-0.80	0.462
	MgSO <sub>4</sub> concentration * Temperature	3.763	6.17	0.002
	H <sub>2</sub> O <sub>2</sub> concentration * Temperature	1.645	2.70	0.043
S = 1.220 R-Sq = 98.4% R-Sq(adj) = 95.6%				

positive contribution to the response was made by temperature with the highest coefficient (5.43). The influence of square terms of H<sub>2</sub>O<sub>2</sub> concentration and temperature were also significant. The highest positive contribution to the response was made by the interaction between MgSO<sub>4</sub> concentration and temperature. A very low *P*-value (0.001) and a very high *F* value (34.43) were obtained by ANOVA (data not shown). This result demonstrated that the full quadratic model was well adjusted to the experimental data and the model was highly significant.

$$\text{Hemicellulose yield (\%)} = 20.420 + 3.629 X_4 + 1.210 X_5 + 5.429 X_6 - 0.074 X_4^2 - 1.996 X_5^2 - 2.719 X_6^2 - 0.485 X_4 X_5 + 3.763 X_4 X_6 + 1.645 X_5 X_6 \quad \text{Equation 4}$$

The resulting contour plots are shown in Figure 1-b. The optimal treatment was found to be at 1.0 for *X*<sub>4</sub> (1% MgSO<sub>4</sub> concentration), 0.596 for *X*<sub>5</sub> (6.4% H<sub>2</sub>O<sub>2</sub> concentration) and 1.0 for *X*<sub>6</sub> (60°C temperature). Hemicellulose extraction yield tests were performed at the predicted optimum extraction conditions with four replicates and the maximum yield was observed as

35.6±0.5%. This result is in agreement with the predicted yield value of 31.2%. The lower and higher limits of the model in 95% prediction interval (PI) for hemicellulose yield are given by Minitab as 26.8% and 35.5%, respectively. It can be concluded that the experimental yield (35.6±0.5%) is at the higher limit in 95% PI, which shows the acceptability of the proposed model. As the result of two-phase optimization procedure, optimum crude hemicellulose extraction conditions were determined as 19.3% (w/v) alkaline (KOH) concentration, 1.3 mm particle size, 25.3 h extraction time, 1% (w/v) MgSO<sub>4</sub> concentration, 6.4% (v/v) H<sub>2</sub>O<sub>2</sub> concentration and 60°C temperature. The hemicellulose extraction yield was increased from an average initial yield value of 21.7 to 35.6%, which corresponds to 64% increase compared to the initial conditions. There are a few studies in literature regarding the hemicellulose content of OSW. Heredia-Moreno et al. (1987) reported the hemicellulose composition of olive stones and their woody fractions as 30.5-27.4% and 27.0-35.1% for different varieties, respectively (24). Albuquerque et al. (2004) have determined an average hemicellulose composition of 350.8 g/kg on dry weight basis, which corresponds to 35.08% (w/w) (25). In another

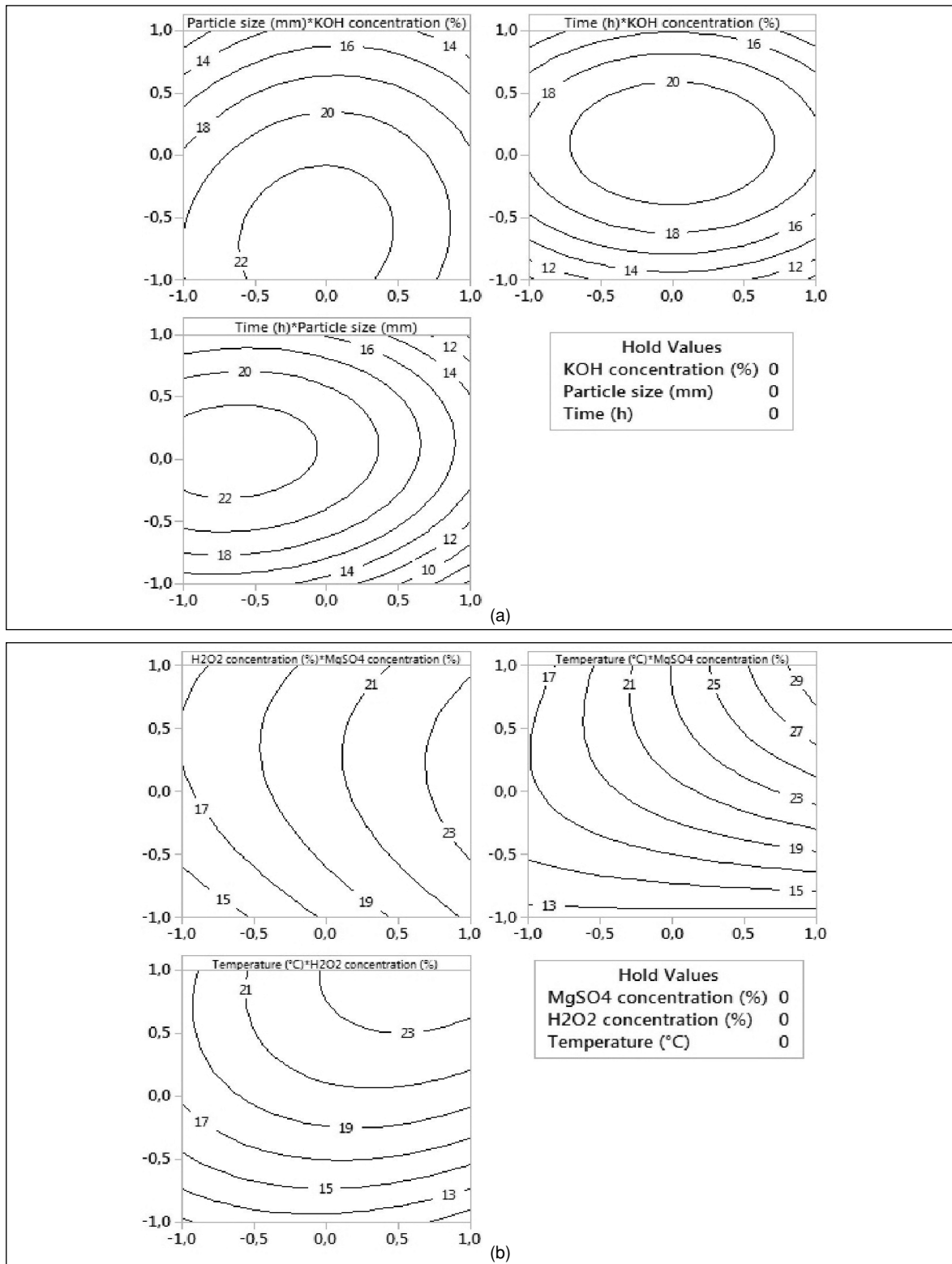


Figure 1. Contour plots (a) showing the effect of KOH concentration, particle size and extraction time on hemicellulose extraction yield of the first optimization phase, (b) showing the effect of MgSO<sub>4</sub> concentration, H<sub>2</sub>O<sub>2</sub> concentration and temperature on hemicellulose extraction yield of the second optimization phase

study, hemicellulose fraction of olive stones was reported as 26.95% (w/w) (26). The extraction yield achieved at optimum conditions was  $35.6\pm 0.5\%$  in the present study, indicating the benefits of process optimization which ensures the achievement of practical limit of extractable hemicellulose yield. Olive oil by-products have several accepted applications in agriculture and in the production of bioactive phenolic compounds, animal feed, food dietary fiber, edible fungi, bioenergy, biofuels, microbial exopolysaccharides, enzymes and biosorbents. The majority of these applications are only described in the scientific literature. The legislations and effective R&D programs will give rise to utilization of olive oil by-products under optimized processes to successfully create a global olive biorefinery (27). From this point of view, the present study fills a gap in the scientific literature and represents a basis for further industrial applications.

Various pretreatment practices have been employed for different lignocellulosic materials so far and chemical and thermochemical techniques are reported as the most promising methods for industrial applications (16). Alkaline pretreatment is one of the widely used chemical pretreatment methods in which the concentration of alkali is an important factor (28). The starting point of the first optimization was 20% (w/w) KOH in this study which provides an average yield of 21.7%. After optimization, KOH dosage was decreased to 19.3% with concurrent increase of the yield. This result demonstrated a possibility of saving in KOH usage with simultaneous increase in the extraction yield. Higher particle sizes led to a reduction in hemicelluloses extraction yield. As expected, decreasing the particle size, hence increasing surface area between solute and solvent caused increasing amount of product (29). Presumably, 1.3 mm OSW particle size already possesses enough large (an optimum) surface area for extraction, so that size reduction does not further increase the hemicellulose yield. Duration of the treatment had an effect with positive response. Increase in temperature showed also a positive effect on the hemicellulose yield and the maximum yield was observed at the highest level of studied temperature range; 60°C. The physical effect of temperature on the extraction was probably the increasing solubility of hemicelluloses with increasing temperature

up to a certain value. At very high temperatures, different sugar compositions are determined in the extraction liquor and the risk for furfural production increases (30). Hemicellulose extraction yield was also highly responsive to  $\text{MgSO}_4$  addition and the maximum yield was observed at 1% (w/w)  $\text{MgSO}_4$  concentration. A similar influence was predicted by Brienzo et al. (2009) during the search for optimum conditions of sugarcane bagasse hemicellulose extraction (13).  $\text{H}_2\text{O}_2$  concentration had also a significant effect ( $P<0.05$ ) with positive response in the current study which is in agreement with the study of Brienzo et al. (2009) (13).

### Colour of hemicelluloses

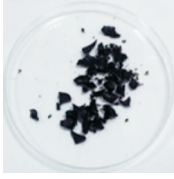
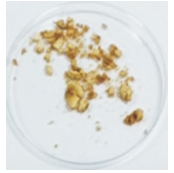
The results of colour readings were summarized in Table 4. In Hunterlab colour scale,  $L^*$  value represents the darkness/lightness of the sample from 0 (black) to 100 (white). The aim of  $\text{H}_2\text{O}_2$  treatment in this study was to achieve light-in-colour hemicelluloses. Accordingly,  $L^*$  value of the OSW hemicellulose was increased from  $1.59\pm 0.24$  to  $31.69\pm 0.28$  after  $\text{H}_2\text{O}_2$  treatment which indicates the success of the delignification by  $\text{H}_2\text{O}_2$  and the lightness of the final product. Doner and Hicks (1997) demonstrated the positive effect of  $\text{H}_2\text{O}_2$  on hemicellulose yield as well as the production of lighter-in-colour hemicelluloses during hemicellulose extraction of corn fiber (17). Fang et al. (1999) reported an optimum alkaline extraction solution which comprises 2%  $\text{H}_2\text{O}_2$  for wheat straw hemicelluloses (31). They aimed to lighten the color of hemicellulose solutions for a successful application in paint industry. Similar influence of  $\text{H}_2\text{O}_2$  on the colour of hemicelluloses was observed in the present study. Hemicelluloses extracted with 8%  $\text{H}_2\text{O}_2$  (v/v) containing solutions were tannish, where hemicelluloses obtained at initial conditions were black in colour. The change in colour was also represented by the great increase in  $L^*$  value of the  $\text{H}_2\text{O}_2$  treated hemicelluloses in compare to the untreated samples. The  $\text{H}_2\text{O}_2$  treatment has provided light-coloured hemicelluloses, for potential utilization in certain food packaging applications and/or in paint industry.

### ACKNOWLEDGEMENTS

This work was partially supported by Karamanoglu Mehmetbey University [BAP-02M14].



Table 4. The changes in colour of extracted hemicelluloses after H<sub>2</sub>O<sub>2</sub> treatment

Hemicellulose	L*	a*	b*
A 	1.59±0.24	0.97±0.26	1.20±0.28
B 	31.69±0.28	-2.90±0.04	5.49±0.04

Data are presented as mean ± standard deviation for n=4

(A) Hemicellulose obtained at the optimum conditions of the first optimization phase (19.3% KOH concentration, 1.3 mm particle size and 25.3 h extraction time at room temperature).

(B) Hemicellulose obtained at the optimum conditions of the second optimization phase (19.3% KOH concentration, 1.3 mm particle size, 25.3 h extraction time, 1% MgSO<sub>4</sub> concentration, 6.4% H<sub>2</sub>O<sub>2</sub> concentration and 60°C temperature).

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