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Optimization of bleaching conditions for palm oil refining to reduce glycidyl esters and improve quality using response surface methodology

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SUMMARY: The impact of bleaching earth content, temperature, and duration on palm oil quality and glycidyl esters was studied using a central composite design at a pilot scale. Response Surface Methodology (RSM) was employed to optimize the bleaching process. The primary goal was to decrease the red color and peroxide value (PV) in order to align with industry interests, and optimization was focused on low glycidyl esters (GE) and *trans*-fatty acid (*trans*-FA) content levels. Several quality parameters, such as red color, free fatty acids (FFA), PV, p-anisidine value (AnV), TOTOX value, solid fat content (SFC), and fatty acid composition were monitored. Under optimal conditions, the predicted values for red color, PV, GE content, unsaturated fatty acids (UFA), and total *trans*-FA content were 2.4, 0.23 meq O_2/kg oil, 0.50 mg/kg, 49.88%, and 0.11%, respectively. Verification showed minimal variations from predictions, indicating a robust optimization model. The bleaching process demonstrated substantial effectiveness, particularly in removing GE, thus enhancing palm oil refining outcomes.

KEY-WORDS: *Bleaching; Glycidyl Esters; Palm oil; Red Color; Response Surface Optimization.*

RESUMEN*: Optimización de las condiciones de blanqueo para la refinación de aceite de palma para reducir los ésteres glicidílicos y mejorar la calidad utilizando la metodología de superficie de respuesta.* Se estudió el impacto del contenido de tierra decolorante, la temperatura y la duración en la calidad del aceite de palma y los ésteres glicidílicos mediante un diseño compuesto central a escala piloto. Se empleó la Metodología de Superficie de Respuesta (MSR) para optimizar el proceso de blanqueo. El objetivo principal fue reducir el color rojo y el índice de peróxidos (IP) para alinearse con los intereses de la industria, y la optimización se centró en obtener niveles bajos de ésteres glicidílicos (GE) y ácidos grasos *trans* (AG-*trans*). Se monitorearon diversos parámetros de calidad, como el color rojo, los ácidos grasos libres (AGL), el IP, el índice de p-anisidina (AnV), el índice de TOTOX, el contenido de grasa sólida (SFC) y la composición de ácidos grasos. En condiciones óptimas, los valores predichos para el color rojo, el IP, el contenido de GE, los ácidos grasos insaturados (AGI) y el contenido total de AG-*trans* fueron 2,4, 0,23 meq O_2/kg de aceite, 0,50 mg/kg, 49,88 % y 0,11 %, respectivamente. La verificación mostró variaciones mínimas con respecto a las predicciones, lo que indica un modelo de optimización robusto. El proceso de blanqueo demostró una eficacia considerable, especialmente en la eliminación de GE, lo que mejoró los resultados del refinado de aceite de palma.

PALABRAS CLAVE: *Aceite de palma; Blanqueo; Colorante rojo; Ésteres de glicidilo; Optimización de la superficie de respuesta.*

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1. INTRODUCTION

Crude vegetable oils should be refined via chemical and physical techniques to eliminate or reduce the content of undesirable substances in vegetable oils. Products that are free of phospholipids, coloring agents and impurities, such as free fatty acids, unpleasant smells, and oxidation byproducts, are preferred by manufacturers (Shahidi, 2020). Additionally, removing these substances is crucial to improving consumer satisfaction. Whether using physical or chemical refining methods, the primary goal of the bleaching step is to decrease the amount of pigments by utilizing an absorbent substance known as bleaching earth. Along with pigments, some reactants are also adsorbed by the active pores in the bleaching earth. During bleaching, peroxides are expected to decompose into smaller, unpleasant oxidation products such as aldehydes and ketones. The simultaneous adsorption of these secondary oxidation products depends on the bleaching earth's adsorption capacity and the parameters of the bleaching process (Gibon *et al*., 2007). Previous studies have optimized refining conditions to minimize the adverse effects of refining steps on the minor components and quality parameters of different vegetable oils (Hew *et al*., 2020; Kreps *et al*., 2014; Suliman *et al*., 2013; Yoon, 2016). In general, bleaching earth is activated using a strong acid to enhance its adsorption capacity. Although excessive acid is removed after the activation process, any remaining acid in the bleaching earth can break down triglycerides into FFAs, raising the overall free fatty acid content during the bleaching process (Abedi *et al*., 2015). Lipid oxidation is another hazard to avoid during bleaching. Therefore, a vacuum is applied to prevent oxidation and improve oxidative stability (De Greyt, 2012). Producing bleached palm oil involves optimizing the process to avoid unwanted reactions, minimize contaminants, and improve color reduction.

Palm oil, an extremely versatile fruit oil with many different properties and functions, is one of the most widely used vegetable oils in food formulations. The global palm oil production was around 77.58 million metric tons in the marketing year 2022/23, increasing from approximately 72.96 million metric tons in 2020/2021. Indonesia and Malaysia were the biggest palm oil exporters (United States Department of Agriculture Foreign Agricultural Service, 2023). Typically, refined bleached palm oil is imported from producing nations and is referred to as RBD, which stands for refined, bleached, and deodorized (Ramli *et al*., 2011). However, during prolonged shipping, specific quality properties may deteriorate due to oxidation and other chemical reactions. Therefore, it is necessary to perform a secondary refining process to produce final products. On the other hand, palm oil has the potential to have widely-known process contaminants, namely 3-monochloropropane-1,2-diol esters (3-MCPDE) and glycidyl esters (GE), due to their formation when elevated temperatures are applied during refining. Since GE has potential genotoxic carcinogenic effects, according to an EFSA report released in 2016, the European Commission strictly limited the maximum GE level to 1 mg/kg and 3-MCPDE to 2.5 mg/kg for palm oils (EFSA CONTAM Panel, 2016; European Commission, 2020). Avoiding and reducing precursors in raw materials and modifying the refining process are effective strategies for minimizing the formation of 3-MCPDE and GE in edible oils. Numerous researchers have studied the adsorption of 3-MCPDE and GE using various adsorbents, including zeolite, magnesium silicate, white clay, and activated carbon (Cheng *et al*., 2017; Restiawaty *et al*., 2021). Research on reducing GE in vegetable oils shows that activated bleaching earth can effectively eliminate GE from vegetable oils (Hew *et al*., 2021; Hew *et al*., 2020).

The positive and negative aspects of the bleaching process should be investigated using an optimization approach; thus, Response Surface Methodology (RSM) is used in many studies to make proper evaluations. This approach creates mathematical models that clarify the relationship between various factors and their responses. To achieve a suitable signal for optimization using Response Surface Methodology (RSM), several experimental designs can be chosen. Among these, the Central Composite Design (CCD) stands out as an effective option since it includes multi-level full factorial designs with axial and star points, yielding accurate results without the need for extensive experimental trials (Deshmukh and Naik, 2015; Hew *et al*., 2021).

This study aimed to investigate the impact of bleaching conditions, such as the amount of bleaching earth used, temperature and bleaching duration on the quality of palm oil. Additionally, the pilot-scale bleaching process parameters were modeled. The study discussed the effects of the bleaching process on the quality properties of palm oil. The bleaching process parameters were optimized to achieve a high-quality palm oil, with reduced red color and peroxide value (PV) and low levels of *trans* fatty acids content and GE. The optimization approach was validated, and the validation results were compared with predicted values.

2. MATERIALS AND METHODS

Palm oil was imported from Malaysia in a standard form, RBD (neutralized, bleached, and deodorized). All bleaching trials and analyses were conducted in the research and development center of Kerevitaş - Kurtköy plant, an industrial edible fat and oil producer in İstanbul, Turkey.

Before the bleaching runs, initial red color, FFA content, PV, AnV, TOTOX value, SFC, GE content and fatty acid composition were determined and discussed in detail.

2.1. Bleaching of palm oil

Bleaching runs were performed using a pilot scale Multi-Purpose Reactor (MPR) (Desmet Ballestra – L2603, Zaventem, Belgium), integrated with an oil temperature control unit (Tool-Temp – TT-380 ATEX, Sulgen, Switzerland), a vacuum pump (Edwards – RV8 ATEX 2C, West Sussex, England) and a filter apparatus (Desmet Ballestra – L2616, Zaventem, Belgium). First, 10 kg of palm oil were heated to 60 °C in a water bath and transferred into the reaction tank with the help of the system vacuum. Palm oil samples were heated and kept at the bleaching temperature for 30 min to remove moisture, as defined in the experimental plan. The vacuum pump reduced the pressure, and the gauge pressure level was kept constant at 60 mbar for all bleaching runs. Bleaching earth was added at the determined amount in the experimental plan to start the bleaching run. The palm oil was stirred continuously at 350 rpm in the reactor for a determined duration. Once the reaction duration finished, the vacuum was decreased by applying nitrogen gas into the reactor. The bleached oil was filtered using a pilot scale press filter and collected into a small tank. The samples acquired after every bleaching run were collected, tightly closed, and stored at -18 °C until analyses. For each bleached sample, the following parameters were measured: peroxide value (PV), p-anisidine value (AnV), free fatty acid (FFA) content, red color, solid fat content (SFC), glycidyl ester (GE) content, and fatty acid composition.

2.2. Chemical analyses

Tintometric red values were measured using an AOCS Tintometer with ¼″ cell (Lovibond, UK) according to the AOCS method Cc 13b-45 and reported as red color (AOCS, 2009). Free fatty acid (FFA) content was measured by titration of the sample with an ethanolic potassium hydroxide solution according to the AOCS method (Ca 5a-40) and expressed as percentages in oleic acid. Peroxide values (PV) were determined via titration of the oxidized potassium iodide using sodium thiosulphate. Results were represented as milliequivalents of oxygen per kilogram according to the AOCS method Cd 8-53. The p-Anisidine value (AnV) was determined according to the spectrophotometric determination of aldehydes at 350 nm as given in the AOCS method Cd 18-90. The total oxidation (TOTOX) value was calculated using the formula TOTOX=2 x (PV)+(AnV). Solid fat content (SFC) values were determined using the IUPAC method 2.150. Samples were heated and kept for 15 minutes at 80 °C and 30 minutes at 60 °C. Then, the samples were conditioned at 0 °C for 30 mins. The solid fat content in the samples was measured using a Minispec MQ20 NMR Analyzer (Bruker, Massachusetts, USA) at 10, 20, 30 and 35 °C after keeping samples at a constant temperature for 30 mins. Fatty acid methyl esters (FAME) were prepared through cold methylation and separated on a capillary gas-liquid chromatography column with a highly polar stationary phase according to chain length, degree of unsaturation, geometry and position of the double bonds according to the AOCS method Ce 1h-05.

The quantification of glycidyl esters (GE) as bound glycidol was carried out using the DGF standard method C-VI 18 (10) by GC-MS. The oil sample was spiked with 1,2-Bis-palmitoyl-3-chloropropanediol-d5 internal standard (TRC Inc, Canada) and transesterified using a sodium hydroxide solution. After liquid-liquid extraction using an iso-hexane - diethyl ether-ethyl acetate mixture, the sample was derivatized using phenylboronic acid (Sigma-Aldrich), dried under nitrogen gas and dissolved in isooctane before injection into the GC-MS (Thermo Scientific, USA). The same procedure was repeated using a sodium bromide solution instead of a sodium chloride solution to stop transesterification. The quantitative difference between the two repeats was multiplied by the transformation factor, and this value represented the GE content of the

sample. Each chemical used in analyses was obtained from Merck at suitable purity levels. All analyses were duplicated, and measurements were taken in triplicate to ensure the accuracy of the results.

2.3. Experimental design and statistical analysis

The experimental plan was developed via the central composite design for three independent variables with three levels according to RSM, consisting of 20 bleaching runs with varying amounts of bleaching earth (0.5-1% of oil), temperature (95-105 °C) and duration (30-60 min). Boundary conditions were extended with a constant alpha (±1.68) as a multiplier to ensure the rotatability of the design space. Design boundaries together with ±alpha extreme conditions are given in Table 1. The complete experimental design of bleaching runs together with results are shown in Table 2. The upper and lower limits of these three independent variables of the bleaching process were determined according to previous works and experiences of the refinery (Hénon *et al*., 1999; Hrastar *et al*., 2011; Yoon, 2016). Statistical evaluations, model development and optimization were carried out using Design Expert ® for Windows version 11.0 (StatEase, Inc., Minneapolis, MN, USA). A second-order polynomial equation was developed to determine the response model's coefficients and their standard errors and significance, as given in Equation 1.

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j X_j + \sum_{j=1}^{k}\beta_{jj} X_j^2 + \sum\sum_{i<j}\beta_{ij} X_i X_j \quad \text{(Equation 1)}$$

TABLE 1. Upper and lower limits of bleaching parameters according to Central Composite Design

Factor	Name	Unit	-alpha	Minimum (Code: -1)	Center (Code: 0)	Maximum (Code: +1)	+alpha
A	Bleaching Earth	% (of oil)	0.33	0.50	0.75	1	1.17
B	Temperature	°C	91.59	95	100	105	108.41
C	Duration	min	19.77	30	45	60	70.23

TABLE 2. Quality characteristics of bleached palm oil samples obtained via central composite experimental design

	Factors			Responses									
Run	Earth (%)	Temp. (°C)	Time (min)	FFA (%)	Red Color	PV	AnV	TOTOX	GE	SFC @10°C	SFC @20°C	SFC @30°C	SFC @35°C
Init				0.22	3.4	6,46	3.72	16.64	4.15	53.6	25.86	8.56	5.76
1	0.5	95	30	0.21	2.8	0.10	8.69	8,89	0.38	53.20	25.81	8.62	5.77
2	1	105	30	0.2	2.6	0.55	9.64	10,74	0.52	54.00	25.90	8.80	5.83
3	1	95	60	0.18	2.8	0.87	9.46	11,2	0.39	53.80	26.20	9.00	5.88
4	0.5	95	60	0.17	2.8	1.00	9.80	11,8	0.57	53.65	24.98	8.54	6.00
5	0.75	100	70.23	0.17	2.7	1.13	10.28	12,54	0.47	53.90	24.90	8.41	6.01
6	0.75	100	45	0.16	2.4	0.10	10.73	10,93	0.41	54.20	25.10	8.98	6.24
7	1	95	30	0.16	2.3	0.32	10.23	10,87	0.55	53.00	26.00	8.55	5.80
8	0.75	100	45	0.15	2.4	0.13	9.71	9,97	0.51	53.80	25.13	8.00	5.87
9	0.75	108.41	45	0.17	2.8	0.44	10.96	11,84	0.46	53.77	25.54	8.74	5.66
10	0.75	100	45	0.18	2.5	0.28	10.71	11,27	0.47	53.23	25.97	8.61	5.51
11	0.75	100	45	0.17	2.5	0.42	10.61	11,45	0.42	52.98	25.00	9.12	5.43
12	0.75	100	45	0.18	2.4	0.36	10.81	11,53	0.47	53.15	24.99	8.21	5.74
13	1.17	100	45	0.16	2.6	0.34	11.10	11,78	0.51	53.20	25.30	8.66	5.98
14	0.5	105	60	0.18	2.8	0.55	11.71	12,81	0.56	53.80	26.05	8.70	5.83
15	0.75	100	19.77	0.17	2.6	0.42	10.70	11,54	0.42	53.66	25.83	8.82	5.32
16	0.75	100	45	0.17	2.5	0.17	10.89	11,23	0.52	53.24	25.03	8.24	5.90
17	0.33	100	45	0.19	2.9	0.18	9.15	9,51	0.45	54.20	25.44	8.48	6.00
18	0.5	105	30	0.17	2.9	0.18	11.07	11,43	0.29	54.00	25.34	8.56	6.00
19	1	105	60	0.19	2.6	0.36	10.43	11,15	0.37	53.12	25.70	8.90	5.55
20	0.75	91.59	45	0.19	2.6	0.48	10.44	11,4	0.54	53.00	25.60	8.91	5.79
		max/min*		1.4	1.3	11.3	1.3	1.4	2.0	1.0	1.1	1.1	1.2

*max/min values were calculated by dividing the maximum value by the minimum value of each response measured after bleaching runs

Y is the predicted response, β0, βi, βii, βij are the regression coefficients for the intercept and the linear, quadratic and interaction coefficients, respectively, Xi and Xj are independent variables, and k is the number of independent variables (Myers *et al*., 2011). The models were developed by reducing from the quadratic degree, and highly insignificant coefficients ($\alpha > 0.1$) were eliminated using the back-substitution method. The main terms (amount of bleaching earth, temperature, and duration) were always kept in the models since those were the primary terms for the hypothesis to sustain the logical expression capability of the regressions. During model reduction, the correct hierarchical structure of the model was maintained (Özdikicierler *et al*., 2016; Zulkurnain *et al*., 2013). Besides the significance of the model values and insignificance of the lack of fit, R2, adjusted R2 (adj-R2), predicted R2 (pred-R2), and adequate precision (APrec) were used for the statistics of the fit for the model (Balasubramani *et al*., 2015; Myers *et al*., 2011). After model development, the ANOVA of the model was evaluated to determine the effect of the factors on responses. P-values less than 0.05 indicated that the model terms were significant. The fitted models for each response were estimated and represented as equations with coded coefficients where the lowest level of a parameter was coded as -1, and the highest level was coded as +1. All levels in between were calculated according to this scale. All responses were measured with three replicates.

Numerical optimizations were applied using only responses with proper model statistics to determine the optimum amount of bleaching earth, temperature, and duration. Optimization aimed at low red color and low PV. The predicted values by RSM were validated by repeating the steam distillation operations under optimum conditions. The results of the three different validation runs and predicted values of the responses were compared. The results of the validation runs within the prediction interval (95%) of the optimization model were used for optimization verification (Myers *et al*., 2011).

3. RESULTS AND DISCUSSION

3.1. Effect of bleaching on the quality characteristics of palm oil

Table 2 represents pre- and post-bleaching results of initial chemical analyses and palm oil SFC percentages. Initial quality parameters and the mean values from all bleached palm oil experimental design results are given in Table 3. The primary purpose of the bleaching operation is to reduce coloring bodies. A significant reduction from 3.4 to 2.63 was observed in tintometric redness after bleaching runs for palm oil. Although FFA content was not affected by bleaching, PV was significantly reduced from 6.46 to 0.42 meq O_2/kg oil. During the degradation of peroxides, light molecular weight substances such as aldehydes are formed, which contributed to an increase in AnV. Thus, AnV increased to 10.36 from 3.72 after bleaching. At the same time, the TOTOX value decreased from 16.64 to 11.19 since PV and AnV are variables used in the calculation of TOTOX.

TABLE 3. Quality criteria, GE content and SFC values before and after bleaching of palm oil

	Red Color	FFA (%)	PV (meq O_2/kg oil)	AnV	TOTOX	GE (mg/kg)	SFC 10°C	SFC 20°C	SFC 30°C	SFC 35°C
Initial	3.4 a (0.1)	0.22 a (0.00)	6.46 a (0.03)	3.72 a (0.04)	16.64 a (0.48)	4.15 a (0.05)	53.6 a (0.37)	25.86 a (0.36)	8.56 a (0.19)	5.76 a (0.69)
Bleached	2.63 b (0.04)	0.18 b (0.00)	0.42 b (0.06)	10.36 b (0.16)	11.19 b (0.20)	0.46 b (0.02)	53.55 b (0.09)	25.49 a (0.09)	8.64 a (0.06)	5.81 a (0.05)

* Standard errors are given in parenthesis. Bleached oil results are the mean values of all bleaching runs.

Some earlier studies indicated a reducing effect of bleaching on 3-MCPD content (Franke *et al*., 2009; Ramli *et al*., 2011). Newer studies revealed that the reduction is much more significant in the GE content of vegetable oils during bleaching (Hew *et al*., 2020; Oey *et al*., 2020; Shimizu *et al*., 2012). According to the results given in Table 3, GE was reduced from 4.15 mg/kg to 0.46 mg/kg, which is lower than the legal limit of 1mg/kg. Some previous studies have discussed a significant reduction in GE content due to adsorption. The GE adsorption activity of acid-activated bleaching earth was also reported for rice bran oil and palm oil, where GE content decreased below 0.1mg/kg from 5.4 mg/kg after bleaching (Shimizu *et al*., 2012). The change in SFC at 10, 20, 30 and 35 °C were measured as 53.6, 25.86, 8.56 and 5.76 %, respectively, and did not change significantly after the bleaching runs.

The SAFA, MUFA, PUFA, UFA, and *trans*-FA contents in the bleached palm oils were calculated using the fatty acid compositions in Table 4. A slight, statistically insignificant increase in SAFA content from 50.01 to 50.39% and a decrease in UFA content from 50.03 to 49.76% were found (Table 5). Also, MUFA, PUFA and *trans*-FA did not change significantly after bleaching runs, when the average values for all runs were considered.

TABLE 4. Fatty acid composition and UFA, PUFA, MUFA, SAFA percentages of bleached palm oil samples

Run	C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1_tr	C18:1_cis	C18:2_tr	C18:2_cis	C18:3	C20:1	C20:0	C22:0	C24:0	SAFA	UFA	MUFA	PUFA	*Trans*-FA
Init.	0.20	0.92	44	0.14	0.10	4.33	0.05	40.1	0.07	9.1	0.36	0.11	0.34	0.22	0.06	50.01	50.03	40.4	9.2	0.12
1	0.19	0.96	44.76	0.19	0.11	4.30	0.04	39.30	0.04	8.98	0,38	0.17	0.33	0.21	0.08	50,75	49.25	39.70	9,13	0.08
2	0.21	1.00	44.38	0.18	0.10	4.26	0.05	39.63	0.03	9.08	0,40	0.15	0.30	0.20	0.07	50,35	49.65	40.01	9,21	0.08
3	0.17	0.95	44.10	0.10	0.09	4.26	0.05	40.02	0.08	9.10	0,33	0.15	0.34	0.22	0.08	50,04	49.96	40.32	9,23	0.13
4	0.20	0.95	44.50	0.14	0.09	4.31	0.04	39.52	0.04	9.12	0,29	0.15	0.36	0.23	0.09	50,55	49.45	39.85	9,27	0.08
5	0.18	0.98	44.41	0.19	0.11	4.40	0.05	39.47	0.09	9.10	0,38	0.15	0.36	0.19	0.09	50,52	49.59	39.86	9,26	0.14
6	0.21	0.99	43.84	0.18	0.11	4.32	0.03	40.12	0.07	9.07	0,36	0.12	0.34	0.20	0.00	49,90	50.12	40.45	9,24	0.10
7	0.19	0.94	43.39	0.19	0.09	4.25	0.05	39.97	0.06	9.12	0,34	0.15	0.33	0.18	0.07	49,28	50.03	40.36	9,27	0.11
8	0.22	0.97	44.12	0.11	0.10	4.33	0.05	39.89	0.04	9.09	0,34	0.14	0.35	0.17	0.08	50,16	49.84	40.19	9,27	0.09
9	0.18	0.98	44.58	0.15	0.11	4.26	0.04	39.55	0.01	9.19	0,34	0.11	0.36	0.09	0.07	50,45	49.53	39.85	9,33	0.05
10	0.17	0.94	44.10	0.17	0.11	4.30	0.05	39.94	0.07	9.02	0,37	0.15	0.36	0.20	0.08	50,07	49.93	40.31	9,18	0.12
11	0.20	0.97	44.13	0.14	0.10	4.24	0.05	39.94	0.05	9.00	0,38	0.16	0.38	0.21	0.08	50,13	49.87	40.29	9,15	0.10
12	0.21	0.96	43.80	0.11	0.11	4.26	0.05	39.54	0.05	9.55	0,31	0.18	0.37	0.18	0.08	49,78	49.96	39.88	9,72	0.10
13	0.17	1.00	44.30	0.16	0.10	4.33	0.05	40.04	0.06	9.00	0,39	0.16	0.35	0.16	0.08	50,31	50.03	40.41	9,17	0.11
14	0.18	1.01	43.92	0.19	0.10	4.27	0.03	39.82	0.08	9.05	0,41	0.18	0.35	0.20	0.08	49,93	49.91	40.22	9,20	0.11
15	0.20	0.99	44.52	0.17	0.10	4.23	0.05	39.10	0.08	9.39	0,35	0.14	0.36	0.22	0.08	50,52	49.47	39.46	9,58	0.13
16	0.22	0.97	44.00	0.18	0.11	4.26	0.05	40.02	0.05	8.98	0,34	0.14	0.37	0.22	0.08	50,04	49.96	40.39	9,18	0.10
17	0.16	0.95	44.42	0.16	0.10	4.33	0.05	39.50	0.05	9.14	0,34	0.16	0.36	0.20	0.08	50,42	49.58	39.87	9,32	0.10
18	0.21	1.02	44.10	0.17	0.10	4.31	0.05	39.80	0.07	9.01	0,39	0.15	0.34	0.23	0.08	50,21	49.79	40.17	9,16	0.12
19	0.19	0.98	44.25	0.16	0.10	4.32	0.05	39.78	0.06	9.01	0,36	0.19	0.33	0.18	0.08	50,25	49.75	40.18	9,15	0.11
20	0.20	0.94	44.50	0.14	0.11	4.33	0.05	39.30	0.02	9.22	0,38	0.18	0.36	0.20	0.08	50,53	49.45	39.67	9,38	0.07
max/min	1.4	1.1	1.0	1.9	1.2	1.0	1.7	1.0	9.0	1.1	1.4	1.7	1.3	2.6	-	1.0	1.0	1.0	1.1	2.8

TABLE 5. SAFA, MUFA, PUFA, UFA and *Trans*-FA contents in palm oil before and after bleaching operations

	SAFA	**MUFA**	**PUFA**	**UFA**	***Trans*-FA**
Initial	50.01 a (0.69)	40.4 a (0.43)	9.2 a (0.17)	50.03 a (0.36)	0.12 a (0.01)
Bleached	50.39 a (0.07)	40.07 a (0.06)	9.68 a (0.03)	49.76 a (0.05)	0.10 a (0.07)

* Values in percentages (%) and standard errors were given in parantheses.

The maximum values/minimum values (max/min) were calculated for all responses after bleaching runs and are presented in the last row of Tables 2 and 4. According to the calculations, the highest max/min ratios were calculated for PV (11.3), C18:2_tr (9.0), *trans*-FA (2.8), C22:0 (2.6) and GE (2.0). This outcome implies that these responses were affected by the bleaching parameters more than the rest of the responses in the experimental plan. However, the effect may not be statistically significant if the values are too low, or the change may not be affected by any of the factors evaluated using model ANOVA.

The ANOVA results, fit statistics and model coefficients of the coded factor levels are presented in Table 6 for each model separately. Significant model F-values were obtained for red color, PV, GE, UFA, and *trans*-FA, with an insignificant lack of fit values related to pure error, indicating that the models were well-fitted to the data. Response surface plots showing the factor effects on these models are shown separately in Figure 1. Additional statistical model fitting measures such as actual vs. predicted, Cook's distances, and residual vs. run graphs of red color, PV, GE, UFA, and *trans*-FA models are shown in Figure 2. All valid model statistics are presented in Table 6.

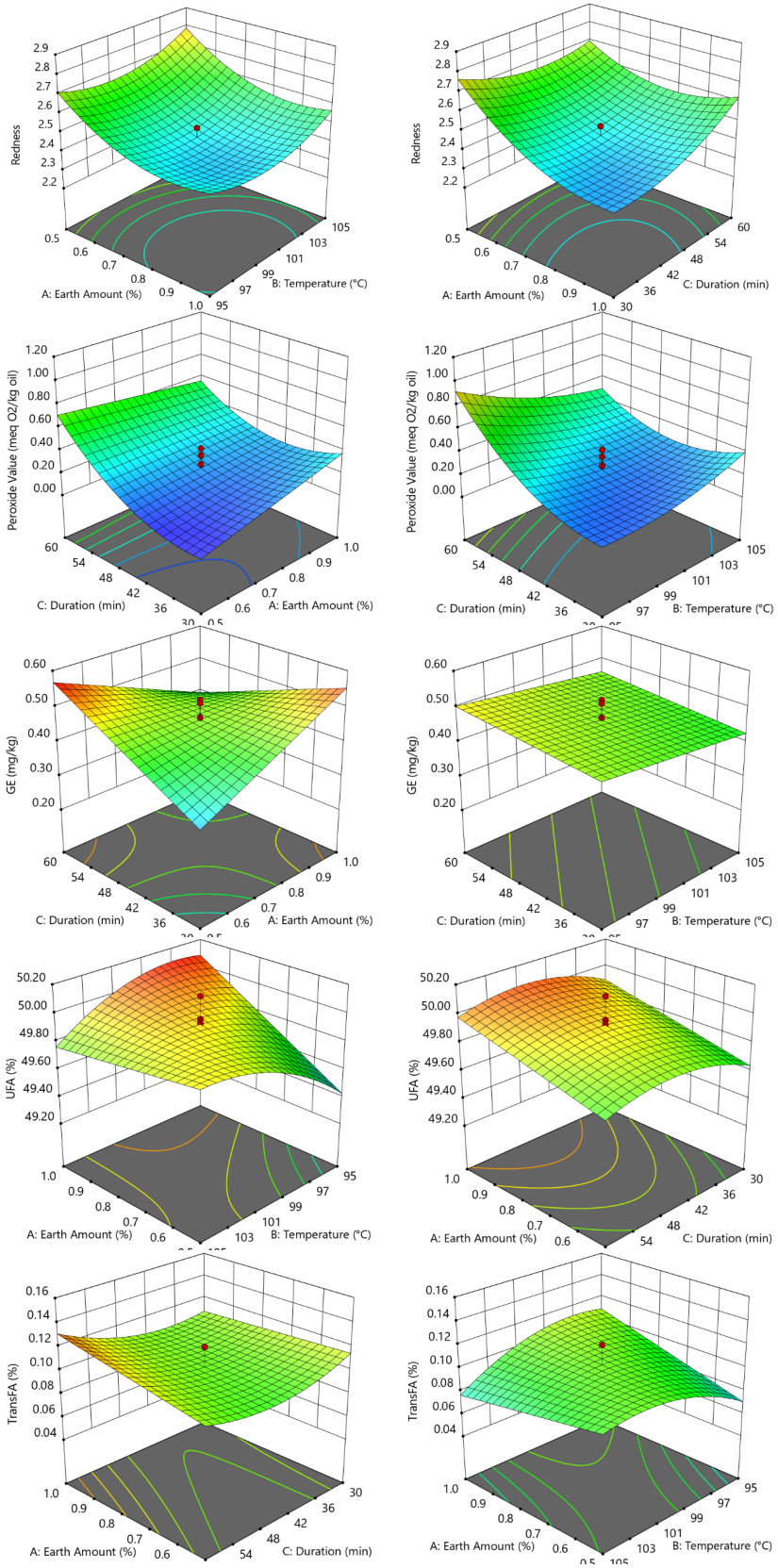

FIGURE 1. 3D response surface graphs plotting significant factors and/or interactions for a) Red Color model, b) PV model, c) GE model, d) UFA model, e) *Trans*-FA model.

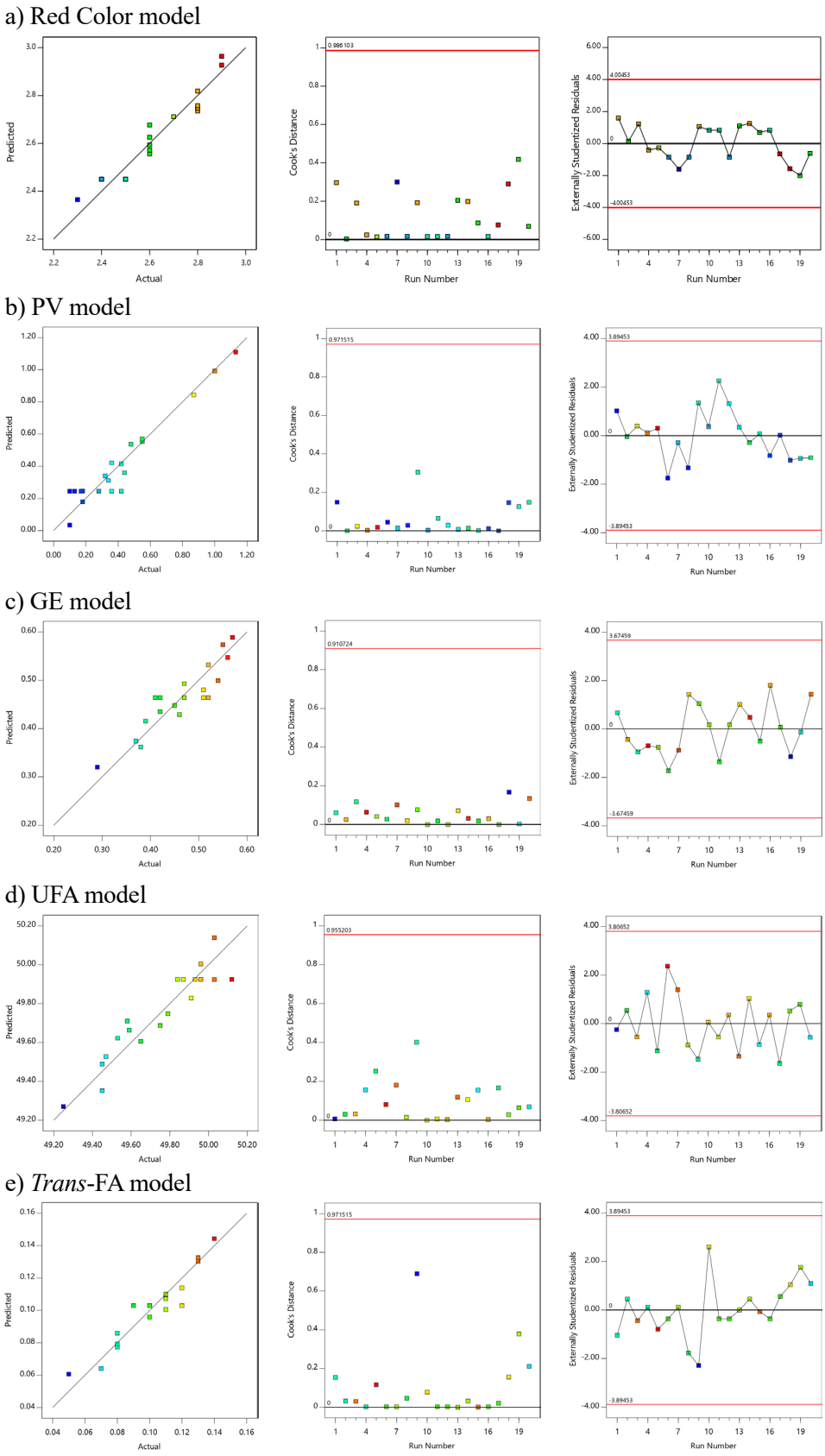

FIGURE 2. Actual vs. Predicted, Cook's distance and residual vs. run graphs of a) Red Color model, b) PV model, c) GE model, d) UFA model, e) *Trans*-FA model.

TABLE 6. ANOVA and fit statistics of Red Color, PV, GE, UFA, and *Trans*-FA models

RED COLOR MODEL

Source	p-value	Coeff. Est.	Fit Statistics	
Model	< 0.0001	2.45	**Min-Max**	2.3-2.9
A-Earth	< 0.0001	-0.1102	**Std. Dev.**	0.0642
B-Temp	0.0449	0.0393	**Mean**	2.63
C-Duration	0.0355	0.0416	**CV**	2.44
AC	0.0070	0.0750	**R²**	0.9267
BC	0.0070	-0.0750	**Adj. R²**	0.8733
A²	< 0.0001	0.1029	**Pred. R²**	0.7122
B²	0.0004	0.0852	**APrec**	13.9148
C²	0.0021	0.0675		
Lack of Fit	0.2923			

PV MODEL

Source	p-value	Coeff. Est.	Fit Statistics	
Model	< 0.0001	0.2450	**Min-Max**	0.1-1.13
A-Earth	0.1533	0.0395	**Std. Dev.**	0.0957
B-Temp	0.0653	-0.0525	**Mean**	0.4190
C-Duration	< 0.0001	0.2068	**CV**	22.83
AC	0.0056	-0.1138	**R²**	0.9309
BC	0.0005	-0.1588	**Adj. R²**	0.8906
B²	0.0143	0.0717	**Pred. R²**	0.8521
C²	< 0.0001	0.1831	**APrec**	17.7991
Lack of Fit	0.9677			

GE MODEL

Model	< 0.0001	0.464	**Min-Max**	0.29-0.57
A-Earth	0.3171	0.0096	**Std. Dev.**	0.0342
B-Temp	0.0399	-0.0208	**Mean**	0.4640
C-Duration	0.0840	0.0171	**CV**	7.38
AC	< 0.0001	-0.0963	**R²**	0.8292
			Adj. R²	0.7837
			Pred. R²	0.7325
			APrec	15.6868
Lack of Fit	0.9167			

UFA MODEL

Model	< 0.0001	49.92	**Min-Max**	49.25-50.12
A-Earth	0.0005	0.1279	**Std. Dev.**	0.1023
B-Temp	0.1732	0.0399	**Mean**	49.76
C-Duration	0.1680	0.0404	**CV**	0.2055
AB	0.0001	-0.1988	**R²**	0.8796
B²	0.0003	-0.1306	**Adj. R²**	0.8240
C²	0.0008	-0.1165	**Pred. R²**	0.6929
			APrec	14.3675
Lack of Fit	0.4602			

***Trans*-FA MODEL**

Model	< 0.0001	0.1030	**Min-Max**	0.05-0.14
A-Earth	0.0947	0.0042	**Std. Dev.**	0.0085
B-Temp	0.6710	-0.0010	**Mean**	0.1015
C-Duration	0.0947	0.0042	**CV**	8.35
AB	0.0003	-0.0150	**R²**	0.9069
AC	0.0277	0.0075	**Adj. R²**	0.8526
B²	< 0.0001	-0.0144	**Pred. R²**	0.7289
C²	0.0001	0.0122	**APrec**	15.6142
Lack of Fit	0.7663			

***** p-value smaller than 0.05 indicates a statistical difference. Coefficients are given for coded factors.

TABLE 7. Optimization and validation results of palm oil bleaching parameters

Optimization – low red color, low PV

Optimum parameter levels			Predictions of optimization model*					
Earth Content (%)	**Temperature (°C)**	**Duration (min)**	**Red Color**	**PV**	**GE**	**UFA**	***Trans*-FA**	**Desirability Factor**
0.86	97.45	34.17	2.4 (2.1-2.7)	0.23 (0.05-0.68)	0.50 (0.35-0.65)	49.88 (49.42-50.34)	0.11 (0.07-0.15)	0.874
		Results of validation trials	2.4 ± 0.1	0.24 ± 0.07	0.51 ± 0.05	49.69 ± 0.12	0.12 ± 0.01	

*95% prediction interval levels (min-max) are given in parenthesis.

The primary purpose of bleaching is to reduce coloring and undesirable materials to improve the oil's appearance by decolorizing vegetable oils using an adsorbent. Tintometric redness, a key indicator of refined oil quality, in the unbleached sample was 3.4 and varied between 2.3 and 2.9 in the bleached samples. The red color was significantly reduced in all bleaching runs, with the max/min ratio calculated at 1.3. The R^2, adjusted R^2 (Adj. R^2) and predicted R^2 (Pred. R^2) for the red color model were 0.93, 0.87, and 0.71, respectively, demonstrating a good fit. Peroxide value (PV), another critical measure of oil oxidation, decreased significantly during the bleaching process. ANOVA indicated that both duration and the interaction between bleaching earth content and duration had significant effects on PV. The model's R^2, Adj. R^2, and Pred. R^2 for PV were 0.93, 0.89, and 0.85, respectively, supporting the model's predictive ability.

PV is a good indicator of oxidation since it measures the peroxide content, the primary oxidation product (Shahidi, 2020). The bleaching operation parameters, especially the amount of bleaching earth, were found effective on PV content; therefore, PV was considered a response for optimizing bleaching parameters (Abedi *et al*., 2015; Ortega-García *et al*., 2006). In our study, the PV value was 6.46 meq O_2/kg oil and was reduced between 0.10-1.13 meq O_2/kg during bleaching operations, which makes the max/min ratio 11.3. Based on the ANOVA's p-values, it was discovered that only the bleaching process's duration affected the PV content among the process parameters. Moreover, the interactions between earth and duration, as well as temperature and duration, were significant. Since the bleaching earth content varied between 0.5 and 1% in our study, which was a very close range, a possible effect of earth amount on PV was not observed. The R2, Adj. R2 and Pred. R2 of the PV model were 0.93, 0.89 and 0.85, respectively, showing good prediction capacity of this model. Therefore, the PV response was selected for optimization purposes in our study.

GE (or GEs) are process contaminants mainly formed from diacylglycerol during high-temperature applications such as deodorization. According to EU Commission regulation (2020/1322), the maximum level of GE for vegetable oils is 1 mg/kg. The limit was set to 0.5 mg/kg for the vegetable oils destined for producing baby food and processed cereal-based food for infants and young children (European Commission, 2020). The initial GE content in our study was 4.15 mg/kg and was reduced between 0.29 and 0.57 after bleaching operations, which can be considered safe for consumption according to the specified EU legislation. GE was appropriately modeled in our study, and R2, Adj. R2 and Pred. R2 were 0.83, 0.78 and 0.73, respectively. APrec value was 15.69, which is higher than 4, showing the model has a good signal-to-noise ratio. Only temperature was found to be statistically significant for GE content, with a p-value of 0.0399, which is close to the significance threshold of 0.05. The interaction between earth content and duration also had a significant effect, suggesting that increased bleaching earth content combined with longer durations enhanced GE removal (Hew *et al*., 2020; Shimizu *et al*., 2012). In addition to this information, the positive impact of duration on GE mitigation was not recorded when a low amount (lower than 0.6%) of bleaching earth was used, according to our results.

UFA content was calculated from the fatty acid composition obtained by summing MUFA and PUFA. The UFA content in vegetable oils is a significant factor for well-known positive health claims. For instance, a high percentage of MUFA, mainly oleic acid, is a main factor in determining the nutritional value of the oil as it reduces the risk of atherosclerosis and protects against different cancers (El Riachy *et al*., 2019). UFA content is always considered beneficial to human health (Kiritsakis and Shahidi, 2017). According to the results, the UFA content was affected significantly by bleaching earth content, whereas UFA content increased directly proportional to the amount of earth, especially at low temperatures (95 °C). The unsaturated fatty acid (UFA) content, while statistically significant, showed minimal variation across bleaching runs, so it was excluded from the optimization process. However, the UFA model still showed good fit statistics ($R^2 = 0.88$, Adj. $R^2 = 0.82$, Pred. $R^2 = 0.69$), indicating reliable predictions.

Deodorization contributes to the formation of *trans* isomers in vegetable oils (MPOB, 2016; Ortega-García *et al*., 2006). Palm oil initially contained 0.12% *trans*-FA, which was reduced to an average of 0.10% after bleaching. Although this reduction was not statistically significant, some bleaching runs showed a drastic reduction in *trans*-FA content to 0.05%. According to ANOVA, interactions between bleaching earth content and both temperature and duration were significant, indicating that higher bleaching earth content at elevated temperatures contributed to a reduction in *trans*-FA. *Trans*-FA content was reduced drastically during some bleaching runs, creating a high max/min ratio. According to model ANOVA, bleaching earth content, temperature, and duration were insignificant, whereas bleaching earth content – temperature and bleaching earth content – duration interactions were significant. Hence, as seen in Figure 1, increasing bleaching earth content reduced *trans*-FA at high temperatures (105 °C). This effect of bleaching earth content on *trans*-FA percentage was not observed at lower temperatures.

3.2. Optimization of bleaching conditions

According to the models obtained, red color, PV, GE and *trans*-FA were used for optimization purposes. The

UFA model was omitted since the absolute difference between bleached palm oils' minimum and maximum UFA content was very low. Although UFA was not included in the optimization approaches, UFA levels were predicted for optimization since the UFA model had good predictive capacity according to the model fit statistics. Based on the models obtained, red color, PV, GE, and *trans*-FA were included in the optimization process. The UFA model was excluded, as the difference in UFA levels among bleached oils was minimal. Numerical optimization focused on achieving low red color and low PV, with 0.86% bleaching earth content, 97.45 °C temperature, and 34.17 minutes of bleaching time determined as the optimal conditions. The predicted values under optimum conditions were 2.4 for red color, 0.23 meq O_2/kg oil for PV, 0.50 mg/kg for GE, 49.88% for UFA, and 0.11% for *trans*-FA. Close red color results were reported by Hew *et al*. (2021), where 2.19 red color was predicted by an optimization model of palm oil bleaching which aims to reduce the content of 3-MCPDE, GE, red color and FFA content (Hew *et al*., 2021). Validation runs performed under optimal conditions confirmed the accuracy of the model, with results close to the predicted values: red color (2.4), PV (0.24 meq O_2/kg oil), GE (0.51 mg/kg), UFA (49.69%), and *trans*-FA (0.12%). These findings indicate that the model is robust and can effectively predict bleaching outcomes under the specified conditions.

4. CONCLUSIONS

Industrial systems are concerned with the organization and effective utilization of available resources in modern manufacturing and process industries to minimize the waste of time, money, materials, and energy. Bleaching is a critical step in the palm oil refining process, where bleaching earth is used to adsorb pigments and other impurities from the oil. This study focused on optimizing the bleaching conditions to achieve reduced red color and peroxide value (PV), while maintaining low levels of glycidyl esters (GE) and *trans*-fatty acids, in line with industry standards. In this study, the bleaching conditions for palm oil were optimized using RSM. Using Response Surface Methodology (RSM), the optimal bleaching conditions were determined as 0.86% bleaching earth content, 97.45 °C temperature, and 34.17 minutes duration. Under these optimal conditions, the predicted values for red color, PV, GE content, unsaturated fatty acid (UFA) content, and *trans*-FA were 2.4, 0.23 meq O_2/kg oil, 0.50 mg/kg, 49.88%, and 0.11%, respectively. Validation of these results confirmed the model's robustness, with validation runs yielding values within the 95% prediction interval. The bleaching process demonstrated considerable efficacy in reducing glycidyl esters, with the final GE content dropping below the legal limit of 1 mg/kg (0.46 mg/kg). This study shows that acid-activated bleaching earth effectively removes GE, and optimization of bleaching conditions significantly improves the quality of palm oil. Given the importance of GE reduction in the edible oil industry, the findings of this study have practical implications for refining processes. Future work could explore further refinements to bleaching conditions or investigate other adsorbents to enhance contaminant removal.

RESEARCH DATA POLICY

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article in tables.

DECLARATION OF COMPETING INTEREST

Conflicts of interest/Competing: There are no conflicts of interest or competing with the results of the presented article.

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N. Başaran: Conceptualization and supervision, review & editing.
F. Altuner: Formal analysis, data curation, and writing – original draft, writing – review & editing
Ö. Anuk: Formal analysis, data curation, and writing – original draft, writing – review & editing
O. Özdikicierler: Data curation, and Writing – original draft, writing – review & editing

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