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Improving the Industrial Practice of Reactive Washing of Cork Stoppers Using a Fractional Factorial Design

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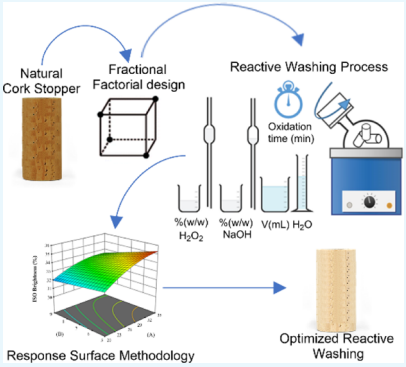
ABSTRACT: Reactive washing (RW) is a key process for disinfecting, purifying, and bleaching of cork stoppers to seal bottles with alcoholic beverages. Excessively severe treatment conditions deteriorate the surface properties of cork stoppers and must be strictly controlled. In this study, the conventional RW of natural cork stoppers was optimized employing a fractional factorial design. The RW variables (H_2O_2 and NaOH concentrations, oxidation time, and washing water volume) were correlated with the final ISO brightness of the stoppers. A three-level and four-factor fractional factorial design within the response surface methodology approach allowed a quadratic model to predict the process response, where the H_2O_2 concentration is the variable with the highest response (ISO brightness), followed by the NaOH concentration. The model obtained was validated, allowing the optimization of the process with savings of 37% in the concentration of H_2O_2 and 33% in the concentration of NaOH and volume of washing water, without deteriorating the final appearance of the stoppers. In addition, the less severe treatment of stoppers under optimized conditions led to less degradation of their surface, thus favoring the receptivity to functional coatings.



INTRODUCTION

Cork is the outer bark extracted from cork oak (*Quercus suber* L.), being produced mainly in the countries of the Western Mediterranean and reaching approximately 200 thousand tons annually.[1−3] The successive layers of suberized dead cells formed by the phellogen of *Q. suber* L. allow the periodical extraction of the cork layer every 9−12 years depending on the geographical area. Not to mention the different composites, cork is widely used in enology as a bottle sealer, occupying the largest market segment of cork products (around 70%). The use of cork as a stopper material is due to its unique physical properties, such as resistance to compression, elasticity and relaxation, controlled permeation, and diffusion of liquids and gases.[3−7] Portugal has a leading position in the production of cork, contributing almost 49% worldwide.[1]

In the transformation process of the oak outer bark into the natural cork stoppers, this material must pass through several industrial steps, where reactive washing (RW) plays a key role in disinfection, surface purification, and appearance (color homogeneity and brightness) of the final product. RW commonly consists in the treatment of stoppers with hydrogen peroxide (H_2O_2) under strong alkaline conditions and increased temperature.[3,8] Hydrogen peroxide evokes the disinfection and degradation of the chromophore structures [double bonds conjugated with the aromatic ring and electron acceptor functional groups (COOH or CHO), quinone structures, among others] on the surface of the cork with an increase in its brightness. The excess of reagents (H_2O_2 and NaOH) is washed away using sequential treatment with sodium hydrosulfate solution to neutralize the alkalinity and with water. All reagents are of high-grade quality according to food safety requirements, whose costs are remarkably superior to those of the corresponding ordinary quality technical products. Quite efficient and cost-effective chlorine-based reagents have been exempted from the practice of RW due to the formation of harmful chloro-organic derivatives, besides causing unpleasant odors and corrupting the flavor of the drinks with which they are in contact.[8−10]

The active specie in cork stoppers' bleaching, hydroperoxide anion (HO_2^-), is formed only under strong alkaline conditions (pH > 11).[11] At the same time, hydrogen peroxide and sodium hydroxide solutions form a relatively harsh reaction system toward main macromolecular cork components.[12] This leads to significant changes in surface properties of cork stoppers, making them more hydrophilic, which in turn affects negatively their subsequent receptivity toward various functional coatings, such as food-grade paraffin and silicon emulsions.[12,13] This coating with paraffin and silicon is essential to control the

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impermeability, sealing, and extraction properties of cork stoppers.[13,14] Thus, the overload of RW reagents is prejudicial not only for economic reasons but also negatively affects the consumption properties of cork stoppers. Depending on the required final stopper brightness, different profiles of reagent addition are adjusted, and the process is evaluated through the measurement of the final ISO brightness or $L \times a \times b$ coordinates. Accordingly, the efficiency of the RW process must be tuned to achieve the lowest reagent consumption without compromising the cork stoppers' final brightness. In this way, ISO brightness is a critical parameter for evaluating the RW efficiency and can be used as a response parameter to the RW process. The optimization of reagents' profiles along RW can be carried out while employing the experimental design techniques, allowing the process analysis and modeling. The expected results contribute to a better understanding of the process variables and to the reduction of its overall costs.[15,16]

The response surface methodology (RSM) is a combination of mathematical and statistical approaches for experimental designs based on the adjustment of a polynomial equation to the experimental data, thus allowing the evaluation of the different factors' effects and seeking the optimal conditions for the desired process.[15−18] For example, if each factor has three levels to analyze and for the four independent variables, the number of practical tests corresponds to 3^4 (or 81) experiments, which can be very time- and labor-consuming. In this way, instead of applying a full factorial design experiment, a three-level and four-factor fractional factorial design can be employed. A fractional factorial design requires fewer experiments than the full factorial and still allows the analysis of the effects of each process variable at different levels as well as their interactions.[19,20] In the case of the RW process in the study, before applying RSM, a well-designed experimental test program is required to determine the response of each factor, which are basically the hydrogen peroxide and sodium hydroxide concentrations, process time, and washing water volume applied.

The main objective of this study was to analyze the effect of operational variables, such as concentrations of hydrogen peroxide and sodium hydroxide, process time, and volume of washing water applied, on the ISO brightness of the cork stopper surface and to evaluate how these interactions between different variables affect the response of interest. To achieve this goal, the RSM approach was used with a fractional factorial design of three levels and four factors, which allowed the optimized process parameters to reach a defined ISO brightness target (33.78%).

RESULTS AND DISCUSSION

Model Equations and Statistical Evaluation. The effects of four process variables (hydrogen peroxide and sodium hydroxide concentrations, oxidation time, and water volume) of RW on the ISO brightness were evaluated using the experimental design of 3^4 fractional factorial experimental with three replications and the analysis of variance (ANOVA) analysis.

Table 1 shows a total of 25 tests generated randomly, which were carried out during the experimental study, as well as the actual ISO brightness values obtained in each assay performed and response values predicted via Design Expert version 11.0.5.0 software. The tests include two replications for run 6 and one replication for run 5.

Table 1. Three-Level and Four-Factor Fractional Factorial Experimental Design and Associated Response [Actual and Predicted ISO Brightness (%)]

					ISO brightness (%)	
run	A	B	C	D	actual	predicted
1	35	9	20	150	33.49	33.86
2	25	7	33	125	33.77	33.79
3	35	9	20	100	33.89	33.66
4	20	9	20	150	32.22	31.84
5	25	7	25	150	33.19	33.51
6	25	9	25	100	32.86	32.59
7	20	5	20	150	32.16	31.99
8	35	7	25	100	34.67	34.68
9	25	5	20	100	33.92	34.08
10	20	9	33	150	32.95	32.99
11	25	9	25	100	32.86	32.59
12	25	7	33	150	34.07	33.95
13	35	5	25	125	35.50	35.31
14	20	7	20	100	31.42	31.60
15	35	5	33	150	34.56	34.80
16	35	9	33	150	33.87	33.54
17	20	5	33	150	33.27	33.34
18	35	7	25	150	34.50	34.28
19	20	9	25	150	31.93	32.30
20	25	7	25	150	33.69	33.51
21	35	9	20	125	33.58	33.65
22	35	9	33	100	32.97	33.26
23	20	5	33	100	33.86	33.65
24	25	9	25	100	32.24	32.59
25	20	5	25	125	32.50	32.60

The predicted ISO brightness (Table 1) ranged from 31.60 to 35.31% depending on the combination of procedural parameters, while the actual ISO brightness has its minimum at 31.42% (run 14) and its maximum at 35.50% (run 13), with an average of 33.36% for the obtained response in the 25 runs. The differences between the actual and the model's predicted values of ISO brightness were relatively small, with an R^2 of 0.9381 (Figure 1A), which indicates that the predicted values agree with the experimental results. The average ISO brightness of natural cork stoppers in RW trials under standard industrial conditions (35% H_2O_2 solution, 9% NaOH solution, and 150 mL of water with a reaction time of 33 min) is 33.78%, which was considered as a target value.

The actual ISO brightness values acquired were fitted to an empirical model, in this case, a quadratic polynomial regression equation based on the coded parameters (eq 1) that correlate the independent variables to the response

$$Y = 33.86 + 1.04A - 0.67B + 0.24C - 0.024D - 0.23AB - 0.37AC - 0.17AD - 0.050BC + 0.32BD + 0.018CD - 0.57A^2 + 0.051B^2 - 0.021C^2 + 0.11D^2 \quad (1)$$

The coefficient of determination (R^2) acquired for the ISO brightness is 0.9381, implying that the regression has a significant value as shown in Figure 1. Figure 1 also shows the residual versus predicted values for ISO brightness, which suggests a uniform distribution.

To evaluate the adequacy of fit of the model toward ISO brightness, ANOVA supplied by Design Expert 11.0.5.0 software was used (Table 2).

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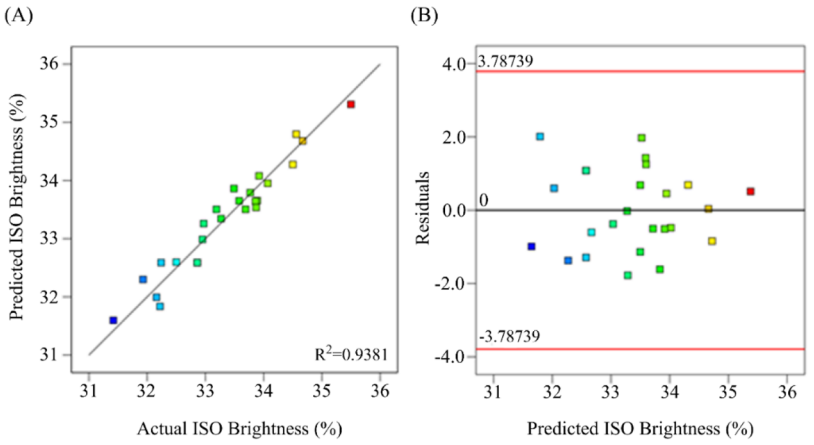

Figure 1. Model diagnostic plots: (A) ISO brightness predicted vs actual plot; (B) residual plot for predicted ISO brightness.

Table 2. ANOVA for ISO Brightness

source	sum of squares	degree of freedom	mean square	*F*-value	*p*-value
model	21.01	14	1.50	10.83	0.0003
residuals	1.39	10	0.1386		
lack of fit	1.00	7	0.1436	1.13	0.5062
pure error	0.39	3	0.1271		

ANOVA is based on the sum of squares determination; thus, the data such as the model sum of squares (21.01), degrees of freedom (14), and mean square (1.50) are the essential parameters for the model evaluation. The results implied that the model has significance since the *p*-value is less than 0.0500 and the *F*-value (10.83) has a value superior to the critical one ($F_{(0.05,14,10)} = 2.87$), which means that the null hypothesis (H_0) is false; that is, at least one of the model parameters b_i is non-zero.[15] Another approach to verify the adequacy of the model toward the ISO brightness actual values is analyzing the lack of fit; this value corresponds to the difference between the model prediction values and the average of the replicated runs performed under the same experimental conditions.[24] Lack of fit *F*-value has a value of 1.13, indicating that the lack of fit is not significant relative to the pure error since it has a value inferior to the critical one ($F_{(0.05,7,3)} = 8.89$); besides, the *p*-value of 0.5062 also characterizes this parameter as non-significant, thus approving the adequacy fitting of the model.

Table 3 presents the coefficients of the model determination statistics with a standard deviation of the predicted model of

Table 3. Coefficient of Determination for the Model

statistics	response: ISO brightness
standard deviation	0.37
adjusted R^2	0.8515
predicted R^2	0.2950
R^2	0.9381

0.37. Through the coefficient of determination analysis, it is shown that the predicted R^2 (0.2950) and the adjusted R^2 (0.8515) have a significant difference; this fact may indicate that factors that have no significance could exist in the model.[25] In this way, Table 4 presents the model coefficient estimate, *F*-value, and *p*-value associated with each parameter of the empirical model.

Table 4. Estimated Coefficients, *F*-Value, and *p*-Value for Each Parameter of the Empirical Model

source	coefficient estimate	*F*-value	*p*-value
A	1.04	92.91	<0.0001
B	−0.67	40.86	<0.0001
C	0.24	4.98	0.050
D	−0.024	0.065	0.80
AB	−0.23	2.97	0.12
AC	−0.37	6.47	0.030
AD	0.17	2.04	0.18
BC	−0.050	0.12	0.74
BD	0.032	5.94	0.035
CD	0.018	0.021	0.89
A^2	−0.57	5.88	0.036
B^2	0.051	0.073	0.79
C^2	−0.021	0.0099	0.92
D^2	0.11	0.24	0.63

The *p*-value consists of the probability, under the assumption of no influence of one of the variables in the response, of obtaining a result equal to or more extreme than what was actually obtained.[26] *p*-Values less than 0.05 indicate that the model terms are significant; in this case, *A*, *B*, *C*, *AC*, *BD*, and A^2 are significant terms in the model.[27] *p*-Values greater than 0.1000 indicate that the model terms are not significant, such as D^2, C^2, B^2, *CD*, *BC*, *AD*, *AB*, and *D*. Thus, the model was reduced in a hierarchical way of the parameters, considering ANOVA, when each of the equation terms of the empirical model was removed. This procedure of the elimination of insignificant terms helps to improve and simplify the model. Equation 2 is the equation of the empirical model; since the parameters that are not significant for the model (D^2, C^2, B^2, *CD*, and *BC*) are excluded, the removal of more parameters does not cause its noticeable improvement

$$Y = 33.95 + 1.03A - 0.65B + 0.23C - 0.020D - 0.24AB - 0.39AC - 0.15AD + 0.30BD - 0.53A^2 \quad (2)$$

Since the model has been changed, all the assumed values obtained until now have been modified, such as the predicted values for each experimental combination of process variables as well as the residuals (Figure 2).

Figure 2 shows that the ISO brightness predicted plotted versus actual experimental values obtained fits well the regression with a coefficient of determination (R^2) of 0.9353,

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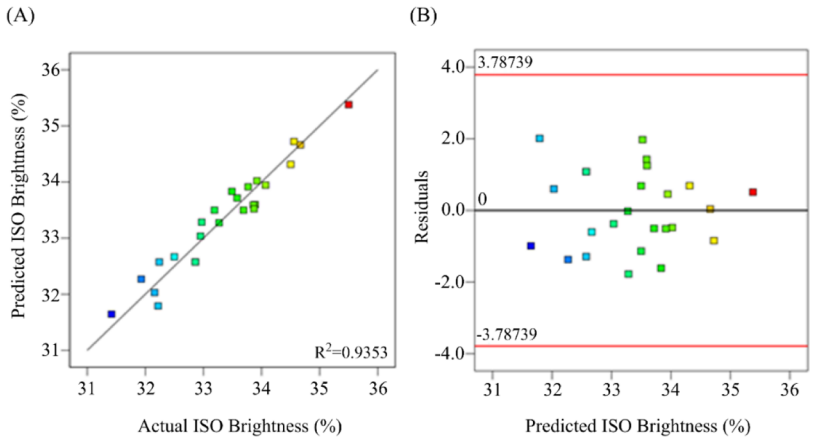

Figure 2. Model diagnostic plots for the reduced empirical model: (A) ISO brightness predicted vs actual plot; (B) residual plot for the predicted ISO brightness.

although it has a slightly inferior value than the extended empirical model (R^2 = 0.9381). The residuals' plot for the predicted ISO brightness is uniformly distributed.

Table 5 presents the ANOVA statistics for the reduced model. The results showed a slight decrease in the model sum of square, and the degree of freedom became 9, which directly implies an increase in the model mean square. The facts that the *F*-value (24.11) has a much greater value than the critical value ($F_{(0.05,9,15)}$ = 2.59) and the *p*-value is less than 0.0001 also confirm the fitting adequacy of the model.[15] The lack of fit *F*-value in the reduced empirical model is inferior (0.70) to that in the initial model (1.13), which shows that this reduction improves the model prediction. Additionally, the *F*-value of the lack of fit (0.70) is lower than the critical one ($F_{(0.05,12,3)}$ = 8.74), which in combination with the *p*-value (0.7172) shows that its value is acceptable in the overall model.

Table 5. ANOVA and Coefficients of Determination for ISO Brightness Using the Reduced Empirical Model

source	sum of squares	degree of freedom	mean square	*F*-value	*p*-value
model	20.95	9	2.33	24.11	<0.0001
residuals	1.45	15	0.0966		
lack of fit	1.07	12	0.0889	0.70	0.7172
pure error	0.38	3	0.1271		

The results presented in Table 6 show that the model reduction also slightly improves the standard deviation by decreasing from 0.37 in the initial model to 0.31 in the reduced one. The coefficient of determination (R^2) also decreases (0.9353), but the predicted R^2 (0.7757) and adjusted R^2 (0.8965) are in good agreement, as the discrepancy between these two values is less than 0.2, which reveals a model with adequate parameters (Table 7).

Table 6. Coefficients of Determination Statistics for the ISO Brightness

statistics	response: ISO brightness
standard deviation	0.31
adjusted R^2	0.8965
predicted R^2	0.7757
R^2	0.9353

Table 7. Estimated Coefficients, *F*-Value, and *p*-Value for Each Parameter of the Reduced Empirical Model (A—H_2O_2 Concentration; B—NaOH Concentration; C—Oxidation Time; D—Washing Water Volume)

source	coefficients estimated	*F*-value	*p*-value
A	1.03	141.23	<0.0001
B	−0.65	63.26	<0.0001
C	0.23	8.07	0.012
D	−0.020	0.071	0.79
AB	−0.24	7.02	0.018
AC	−0.39	17.57	0.00080
AD	−0.15	2.73	0.12
BD	0.30	9.53	0.0075
A^2	−0.53	9.56	0.0074

The data presented in Table 7 also indicate that there are still terms in the model that have no significance. However, the removal of such terms (*AD* and *D*) did not lead to a general improvement of the model. Consequently, the model that was previously considered most suitable for the experimental results of the real ISO brightness is the reduced empirical model described by eq 2.

Effect of Process Variables on the ISO Brightness. The estimated coefficients for each model parameter that resemble the RW process variables from eq 2 (Table 7) indicate that factors *A* and *C* (concentration of the H_2O_2 and the oxidation time, respectively) have a positive effect on the ISO brightness. On the other hand, the *B* factor (concentration of NaOH) has a negative effect. This is understandable since alkalinity favors the formation of chromophores due to the formation of quinone structures with lignin and tannins presenting on the surface of the cork stoppers.[12] Accordingly, the excess of NaOH is strongly prejudicial to the brightness of the stoppers and must be corrected accordingly. In addition, the *D* factor (amount of water in the washing step) has no significant effect on the ISO brightness in the range of the parameter levels examined, but due to the design model, hierarchical rules cannot be removed. Similarly, eq 2 establishes the effects of each interaction studied in the model, which shows that all factors, with exception of BD, have a negative effect on the ISO brightness. With the reduction of the empirical model, the only quadratic effect present in the equation is factor *A*, which has a negative effect on the response.

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The predicted response of the process is represented in a three-dimensional form of the response surface shown in Figures 3–6, plotting the interaction between two variables. Figure 3 shows the effect of *A* and *B* on the ISO brightness at the center level of *C* and *D*.

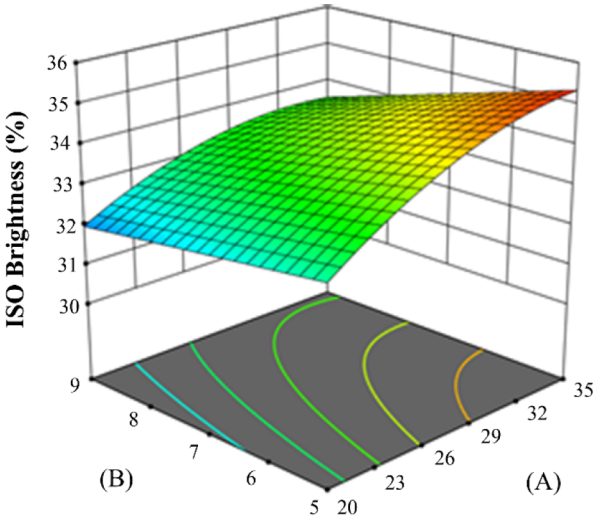

Figure 3. Response surface plot representing the effect of *A* and *B* on the ISO brightness.

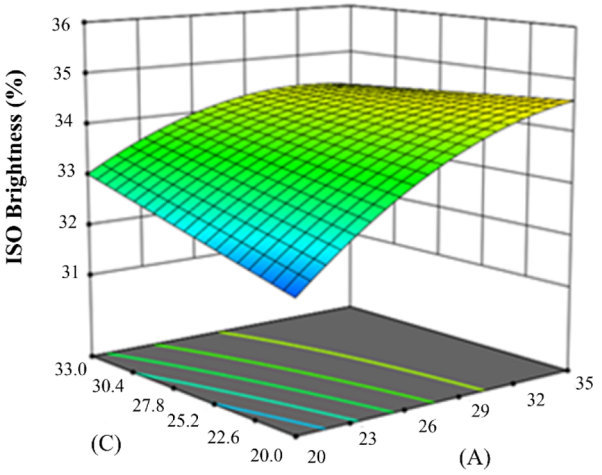

Figure 4. Response surface plot representing the effect of *A* and *C* on the ISO brightness.

Figure 3 also shows that increasing factor A (H_2O_2 concentration) causes an improvement of the ISO brightness. On the other hand, the alteration of factor *B* (NaOH concentration) does not change the ISO brightness in a significant way. This can be explained by the excess of the NaOH concentration in the reaction system. The best value of ISO brightness (35.34%) is obtained when variable A is at the highest level and variable *B* in the lowest one (Figure 3). However, taking into consideration that the target ISO brightness is 33.78%, it is possible to reduce factor *A* in RW while keeping the other two variables (*C* and *D*) in the center point.

Figure 4 shows the effect of *A* and *C* variables on the ISO brightness at the center level of *B* and *D*. It is noteworthy that for the maximum level of *A*, factor *C* has almost no significant effect on the ISO brightness for the levels examined. However,

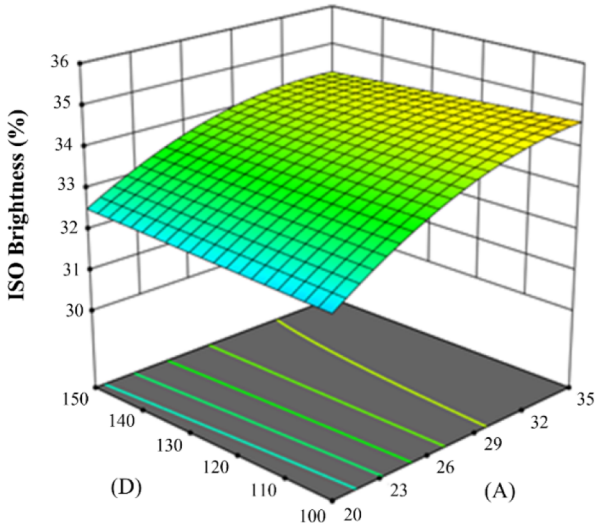

Figure 5. Response surface plot representing the effect of *A* and *D* on the ISO brightness.

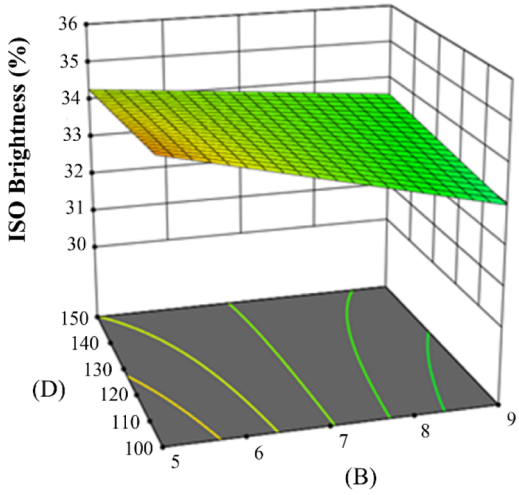

Figure 6. Response surface plot representing the effect of *B* and *D* on the ISO brightness.

if factor *A* is at the minimum level, factor *C* has more influence on the response, and the lowest brightness value (31.78%) is less than the ISO brightness target (Figure 4). Apparently, when the chromophores of the cork do not degrade extensively due to the lack of H_2O_2, their removal from the surface of the stoppers is more dependent on the reaction time (factor *C*).

Figure 5 shows the effects of *A* and *D* factors on the ISO brightness, keeping factors *B* and *C* in the center level. As stated before and confirmed by Figure 5, factor *D* (amount of washing water) has no significant effect on the ISO brightness because it does not cause noticeable changes in the response. If factor *A* is increased to the highest levels examined, the response is improved, but for the lowest levels of *A*, the ISO brightness (32.27%) is less than the target value.

Figure 6 shows the effects of the last interaction present in the model, factors *B* and *D*, while factors *A* and *C* are in the center level. Considering the levels examined, it can be seen that the best value for the ISO brightness is obtained for the lowest levels of *B* and *D* factors. This is in tune with the previous discussion when the lowest level of alkalinity (*B* factor) provoked less formation of chromophores on the

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surface of stoppers and needs less water to wash out the reaction products (*D* factor). The variation of *D* factor for the highest or the lowest level of the *B* factor does not imply any significant change in the response. From the response surface plot shown in Figures 3−6, this empirical model allows the optimization of the RW process.

Model Validation. To validate the model that predicts the ISO brightness after the RW process applied, nine additional tests were performed using different levels of independent variables. The *D* factor was not modified, and it was maintained at the lowest level because this does not have a significant influence on the response. The test results as well as the predicted and the actual responses are presented in Table 8.

Table 8. Conditions of the Validation Tests and Predicted and Actual ISO Brightness

	condition				ISO brightness (%)	
run number	*A*	*B*	*C*	*D*	actual	predicted
1	20	5	20	100	32.90	32.35
2			25		31.00	32.83
3			33		30.00	33.60
4	25	7	20		33.40	33.16
5			25		33.10	33.44
6			33		32.55	33.88
7	35	9	20		32.50	33.60
8			25		32.89	33.48
9			33		33.61	33.29

Figure 7 shows the relationship between the ISO brightness predicted by the model and the respective value obtained

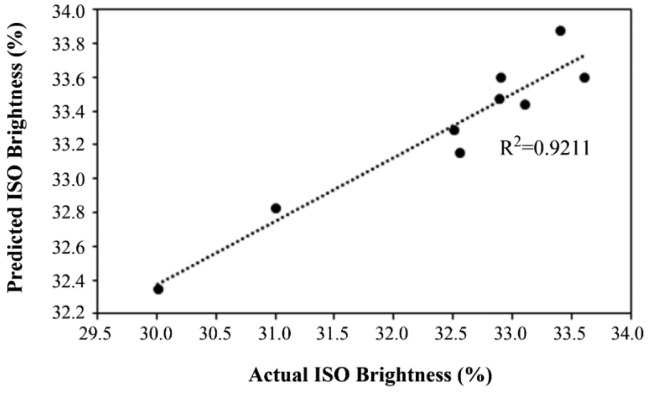

Figure 7. Relationship between the actual and predicted ISO brightness.

experimentally for each of the nine tests. The results obtained clearly indicate that it is possible to validate the model developed since the real experimental values are in accordance with those predicted, with a coefficient of determination (R^2) of 0.9211.

Optimization Results. Using the numerical option of the optimization module of the Design Expert software (version 11.0.5.0), it was possible to optimize the levels of the independent variables to obtain the target ISO brightness of 33.78%. The results obtained showed that natural cork stoppers with a target of 33.78% ISO brightness can be achieved with a value of 22 for variable A (% of H_2O_2 concentration), 6 for variable B (% of NaOH concentration), 33 for variable C (time of the oxidative treatment, min), and the lowest level of 100 for variable D (water consumption for the washing, mL/10 stoppers). This implies significant changes in the profile of the added reagents. Such an approach leads to a significant improvement in the RW process, thus allowing a reduction of 37% for variable A and 33% for variables B and D, respectively, without deteriorating the final quality parameter of natural stoppers (ISO brightness).

The decrease in reagent consumption in the optimized RW caused less degradation of the hydrophobic polymers (primary suberin) on the surface of natural stoppers, in relation to stoppers after the conventional RW.[12] This was confirmed by the free surface energy (γ_s) values and its corresponding polar (γ_s^p) and dispersive (γ_s^d) components of the stoppers treated by optimized RW. The results were much closer in terms of gaining the polarity index (γ_S^P/γ_S) for untreated stoppers than for stoppers treated by conventional RW (Figure 8).

Moreover, the known anisotropy in surface properties of the lateral and top of the natural cork stoppers[12] was leveled out to a significant extent after RW with an optimized reagents' profile. In practice, this means better receptivity of hydrophobic coating formulations (e.g., paraffin emulsion or decorative polymeric formulations) by natural stoppers treated by the optimized RW process than by the conventional RW process.[12] Consequently, optimized RW has not only saved reagents but has also improved the processability of natural stoppers in relation to the targeted coatings.

It is noteworthy that the changes inferred in the reagent load in optimized RW were confirmed in pilot experiments with 3000 stoppers under conditions similar to industrial ones, which clearly corroborates the optimization results obtained in laboratory tests.

CONCLUSIONS

The effect of four RW process independent variables (hydrogen peroxide and sodium hydroxide concentrations, oxidation time, and washing water volume) on the ISO brightness of the natural cork stoppers on a laboratory scale was studied. A three-level and four-factor fractional factorial experimental design and RSM were used to develop mathematical models for ISO brightness response using experimental data and software Design Expert version 11.0.5.0. The experimental results were fit to a second-order polynomial equation, and the model was optimized by elimination of several insignificant factors and validated. The model developed revealed that hydrogen peroxide concentration is the variable that most influences the response (brightness of cork stoppers), followed by sodium hydroxide concentration. Time of the oxidative treatment variable had significance only at relatively low hydrogen peroxide concentrations (variable A). The volume of washing water has no significance in the developed model. This was explained by the need to change the profile of the reagents in relation to the final brightness of the stoppers. By applying the optimized reagent profiles with an ISO brightness target (33.78%), it was possible to obtain a significant improvement in the process in terms of reagent savings, with a reduction of 37% for variable *A* and 33% for variables *B* and *D*, respectively. In addition, the optimized RW conditions, less degrading to the cork surface, revealed an increased potential to improve the receptivity to its functional coatings. The results obtained in the laboratory were

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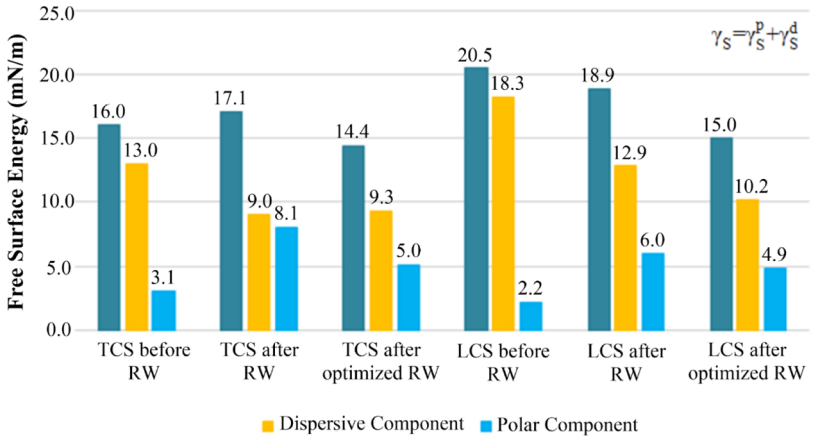

Figure 8. Contribution of polar and dispersive components to the total free surface energy of the lateral (LCS) and top (TCS) of the cork stopper before and after conventional RW and after optimized RW.

later confirmed in tests on a pilot-scale simulating industrial practice.

EXPERIMENTAL SECTION

Natural cork stoppers were supplied by Amorim Cork, S.A. (Santa Maria de Lamas, Portugal). The stoppers were from the same batch, that is, originated from the same industrial processing preceding RW, which reduces the variability of the stopper process before washing. Cork stoppers have a single caliber 49 × 24 mm (length × diameter) and belong to the 1st class (middle class). Hydrogen peroxide, sodium hydroxide, and sodium hydrosulfate, all of which were of food grade and currently used in industry, were also supplied by the company Amorim Cork S.A. (Santa Maria de Lamas, Portugal).

RW Procedure in the Laboratory Scale. The RW process was carried out on a laboratory rotation glass reactor (100 rpm) under controlled temperature (50 °C). In a typical trial, the reagents (NaOH and H_2O_2) were added alternately to the reactor containing 10 natural cork stoppers following the sequence and the timesheet protocol used in the industry, respecting the reagent-to-stopper ratio. These details are not disclosed here for confidentiality reasons. After 5 min of the reagents coming in contact with the stopper surface, they are removed to avoid cork swelling.[8] Once the oxidation step is completed, the stoppers were washed with distilled water. Sodium hydrosulfate solution (2.5% w/w) was added further to neutralize the surface of the stoppers, which were washed again with distilled water. The treated stoppers were dried in a ventilated oven at 40 °C for 1 h. Afterward, the cork stoppers were allowed to stabilize for 24 h before the evaluation of ISO brightness.

ISO Brightness Assessment and Contact Angle Measurements. Brightness is the key parameter to evaluate the efficiency of RW, thus reflecting the stopper appearance. The ISO brightness corresponds to a numerical value of diffuse reflectance (% ISO) with respect to the blue light of wavelength 457 nm. A similar procedure is also commonly used in the pulp and paper industry according to norm TAPPI T 525 om-06. The analysis was performed on the Konica Minolta cm700-d portable spectrophotometer (Konica Minolta; Tokyo, Japan) and was adapted from the internal procedure used in the company Amorim Cork S.A. In general terms, all 10 stoppers used in the assays were evaluated through ISO brightness analysis, performing three random measurements on the top and on the lateral surfaces. In this way, each stopper's ISO brightness value corresponds to the average of six measurements. The final ISO brightness assigned to each test is the ISO brightness mean value of the 10 stoppers used.

Contact angles were measured using an OCA20 of Data Physics Instruments goniometer (Data Physics, Filderstadt, Germany) equipped with a charge-coupled device camera using the sessile drop method with water, formamide, and diiodomethane as probe liquids with known total surface energy and dispersive and polar component values.[12] For each sample, 30 measurements were performed (10 measurements for each solvent used). The contact angle measurements were carried out at room temperature under controlled conditions (21 ± 1 °C, RH 60%) and applying the drop volume of 1 μL with a velocity of deposition of 1 μL/s. Contact angles were measured as a function of time for 60 s and then extrapolated to zero time. The results of parallel measurements were averaged and the standard deviation errors evaluated. The evaluations of the free surface energy of cork (γ_s) and its corresponding polar (γ_s^p) and dispersive (γ_s^d) components were performed using the aforementioned liquid probes based on the Owens−Wendt−Rabel−Kaelble (OWRK)model[12]

$$\left(\frac{1+\cos\theta}{2}\right)\left(\frac{\gamma_L}{\sqrt{\gamma_L^d}}\right)=\sqrt{\gamma_s^p}\times\sqrt{\frac{\gamma_L^p}{\gamma_L^d}}+\sqrt{\gamma_s^d} \quad (3)$$

All measurements of the contact angles were carried out on the surface areas of the natural stopper in the absence of lenticular channels, and the droplet deposition was carefully selected to avoid the interference of the oval shape of the side of the stopper.

Response Surface Methodology. The first task to apply the RSM is to establish the levels for each variable under study (hydrogen peroxide and sodium hydroxide concentrations, oxidation time, and water volume). To complete this task, a series of tests were carried out using the time one-factor-at-a-time methodology. These pre-experimental tests provided information about the influence of each factor in the RW process through the ISO brightness obtained for each test. These assays are not shown in the article as they were not the main objective of this study. Although this methodology gives helpful information about the process, it does not consider the

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possibility of interactions between factors. Thus, the effect of different operating parameters on the RW process was evaluated using a three-level four-factor fractional factorial experimental design approach.[15,16,21,22] The RW procedure variables (*A*, *B*, *C*, and *D*) with their coded and actual levels are presented in Table 9.

Table 9. RW Procedural Variables and Respective Coded and Actual Levels[a]

		real values of coded levels		
variables	type of variable	−1	0	1
A	discrete	20	25	35
B	discrete	5	7	9
C	discrete	20	25	33
D	discrete	100	125	150

[a]−1: factor at a low level; 0: factor at a medium level; +1: factor at a high level.

Each coded variable (*A*, *B*, *C*, and *D*) in the study is associated with one of the procedure variables as hydrogen peroxide concentration (%, w/w), sodium hydroxide concentration (%, w/w), oxidation time (min), and water volume (mL/10 stoppers), respectively. All the variables are discrete, which means that the variable has measurable characteristics and is either finite or countably infinite.[15]

The actual values used for each variable correspond to the combination of all the higher levels assigned for each factor. This experimental design resulted in 25 assays with three replicates. These replications are essential to understand the process variability.[23]

The experimental results were fitted to a second-order polynomial (eq 4). The model characterizes the effects of process variables (*A*, *B*, *C*, and *D*) and their interactions on the response variable *Y* (ISO brightness).

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D + b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC + b_{24}BD + b_{34}CD + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{44}D^2 \quad (4)$$

where *Y* is the predicted response; b_0 is the model constant; b_1, b_2, b_3, and b_4 are linear coefficients; b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, and b_{34} are cross-product coefficients; and b_{11}, b_{22}, b_{33}, and b_{44} are the quadratic coefficients. Statistical Stat-Ease Design Expert 11.0.5.0 software (Stat-Ease Inc., Minneapolis, MN, USA) was used to establish the validity of the models on the basis of ANOVA and coefficient of determination (R^2).

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Author Contributions

D.B. participated in the methodology, investigation, visualization, and writing−original draft. C.S. completed the investigation and visualization. L.C. was in charge of the project administration; supervision, resources, validation, and writing−review and editing. D.E. performed the funding acquisition, conceptualization, supervision, resources, validation, and writing−review and editing.

Notes

The authors declare no competing financial interest.

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