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Optimization and Validation of Rancimat Operational Parameters to Determine Walnut Oil Oxidative Stability

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Abstract: This study was performed to optimize and validate Rancimat (Metrohm Ltd., Herisau, Switzerland) operational parameters including temperature, air-flow, and sample weight to minimize Induction-Time (*IT*) and *IT*-Coefficient-of-Variation (*CV*), using Response Surface Methodology (RSM). According to a Box–Behnken experimental design, walnut oil equivalent to 3-, 6-, or 9-g was added to each reaction vessel and heated to 100, 110, or 120 °C, while an air-flow equal to 10-, 15-, or 20-L·h^{-1} was forced through the reaction vessels. A stationary point was found per response variable (*IT* and *CV*), and optimal parameters were defined considering the determined stationary points for both response variables at 100 °C, 25 L·h^{-1}, and 3.9 g. Optimal parameters provided an *IT* of 5.42 ± 0.02 h with a *CV* of 1.25 ± 0.83%. RSM proved to be a useful methodology to find Rancimat operational parameters that translate to accurate and efficient values of walnut oil *IT*.

Keywords: walnut oil; rancidity; induction time; rancimat; response surface methodology

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1. Introduction

Oxidative deterioration, commonly known as lipid oxidation or rancidity, causes off-flavors, discoloration, unpleasant odors, and decreases edible oil nutritional value. Lipid oxidation is a problem of economic concern to the walnut industry, as it decreases the nuts positive quality characteristics (e.g., flavor, smell, and color) and shortens their shelf life [1]. Walnuts are particularly susceptible to oxidation-induced rancidity because of their high oil content (60 to 70%) and elevated content (~70%) of polyunsaturated fatty acids (PUFA), such as linoleic (56.5–57.5%) and linolenic (11.6–13.2%) fatty acids, and monounsaturated (~21%) fatty acids, mainly composed by including oleic acid (21.0–21.2%) [2,3]. Nuts with lower PUFA content have a longer shelf life [4] like hazelnut and macadamia, which contain around 13.6% and 3.7%, respectively [5].

In order to evaluate rancidity in walnut oil, extracted from fresh walnut kernels, it is necessary to determine the oil oxidative stability. Walnut rancidity in fresh walnut samples is highly variable [6]. Therefore, it is preferred to estimate the oil rancidity, instead of its corresponding fresh ground samples, as oil is a better and more uniform representation of a large batch of walnuts. The quantification of free fatty acids (FFA) and peroxides or peroxide value (PV), p-Anisidine, and test of thiobarbituric acid (TBA) are important measurements that have been used for decades to evaluate oil oxidative stability [1]. However, these tests provide information regarding a temporal fat oxidative state, and rely on the use of toxic solvents [7] and manual titration that may lead to erroneous results [8,9]. Considering that lipid oxidation is a dynamic process, a forward path in directly evaluating rancidity is to use an accelerated oxidation method to determine the lipid's relative oxidative stability, also known as the Induction Time (*IT*), which is defined as the resistance of lipids to be oxidized [9]. The Rancimat equipment is widely used by the edible and pharmaceutical oils industries, as it does not require the use of hazardous materials and intensive labor in comparison to other accelerated oxidation methods, such as the Active Oxygen Method (AOCS Method Cd 12-57) [9,10].

The Rancimat equipment (Metrohm Ltd., Herisau, Switzerland) automatically determines the *IT* by measuring conductivity changes as volatile compounds are generated through an accelerated oxidation method or aging test. The Rancimat accelerated oxidation method consists of exposing an oil sample to a constant high temperature (between 50 and 200 °C), and airflow (between 1 and 25 L·h^{-1}) to guarantee a sufficient oxygen supply to rapidly induce lipid oxidation. Operational parameters are typically established by the Rancimat equipment manufacturer, suggesting that parameters to estimate walnut oil *IT* are 3 g of sample, using a temperature of 110 °C, and an airflow equal to 20 L·h^{-1} [11]. Nonetheless, others studies that have evaluated walnut oil *IT*, using the Rancimat equipment, have used a broad range of sample weights (2.5 to 5 g), temperatures (100 to 110 °C), and airflows (15 to 20 L·h^{-1}) [2,4]. Unfortunately, none of the previous studies including the suggested manufacturer parameters properly describe how the operational parameters were optimized and validated. Therefore, results are widely variable, inconsistent, and incomparable. Therefore, there is a clear need to accurately describe how walnut Rancimat equipment *IT* determination can be optimized and validated, as described in this study. Additionally, there are currently no studies properly describing the effect of differences in the Rancimat operational parameters in the *IT* determination. This study provides an in-depth understanding of this effect, providing valuable information to those in need of properly estimating walnut oil oxidative stability.

Response Surface Methodology (RSM) is a multivariate statistic technique used to develop and improve processes in which several input variables may have an effect in the process performance and output [12]. RSM statistical designs offer quadratic response surfaces, which include central composite, Doehlert matrix, three level full factorial, and Box–Behnken designs. Overall, the Box–Behnken design has proven to be one of the most efficient, as it requires a fewer number of experiments than the minimum required for a complete three level factorial design [13]. By using Box–Behnken experimental design, Donis-González, Guyer [14] successfully optimized the computed tomography image scanning parameters (voltage, current, and slice thickness) to predict chestnut (*Castanea* spp.) internal quality. Rodríguez-González, Femenia [15] also used a Box–Behnken experimental design to resolve the parameters (plant age, pasteurization temperature, and time) that maximized alcohol swelling, water retention capacity, and fat absorption capacity of *Aloe vera* as a polysaccharide-rich food ingredient extract.

Therefore, the objective of this study was to use the RSM statistical technique in combination with a Box–Behnken experimental design to optimize and validate the Rancimat operational parameters (sample weight, temperature, and airflow), and establish a standard for future walnut oil *IT* estimations. The goal was to minimize the *IT* Coefficient-of-Variation (*CV*) within the same oil sample, and report the yielded *IT* measurements. A minimized *CV* within the same oil is reflected as a minimum error in *IT* estimation.

2. Materials and Methods

Figure 1 shows a schematic representation of the methodology followed in this study.

2.1. Walnut Collection, Oil Extraction and Storage

Walnuts (*Juglans regia* L.) var. "Howards" and "Tulare" were collected during the 2017 harvesting season from two walnut hullers/driers located at Meridian and Visalia, California. Walnuts were dehydrated in a six-column convective dryer at 43 °C for 48 h, to achieve an in-shell moisture content of around 6 to 8% wet basis (MC_{wb}), as specified by Khir, Pan [16]. In-shell walnuts were stored in burlap bags at ambient conditions (8–20 °C, and 53–93% relative humidity) for six months, before oil extraction.

Six kg of walnuts were manually shelled using a walnut cracker, and shells were disposed of. Walnut kernels were placed inside Ziploc bags, manually crushed with a hammer, and strained through a 1.70 mm sieve. Forty g batches of the crushed walnut kernels retained on a secondary sieve (710 µm) were placed in a stainless-steel oil extraction cylinder (5.7 cm diameter by 7.6 cm height) to extract their oil. Walnut oil was extracted

using a Carver Press Model 3351 by applying a constant force of 5 metric tons (MT) for 5 min (Carver Inc., Wabash, IN, USA). On average, 15 mL of walnut oil were collected per batch in a stainless-steel pan.

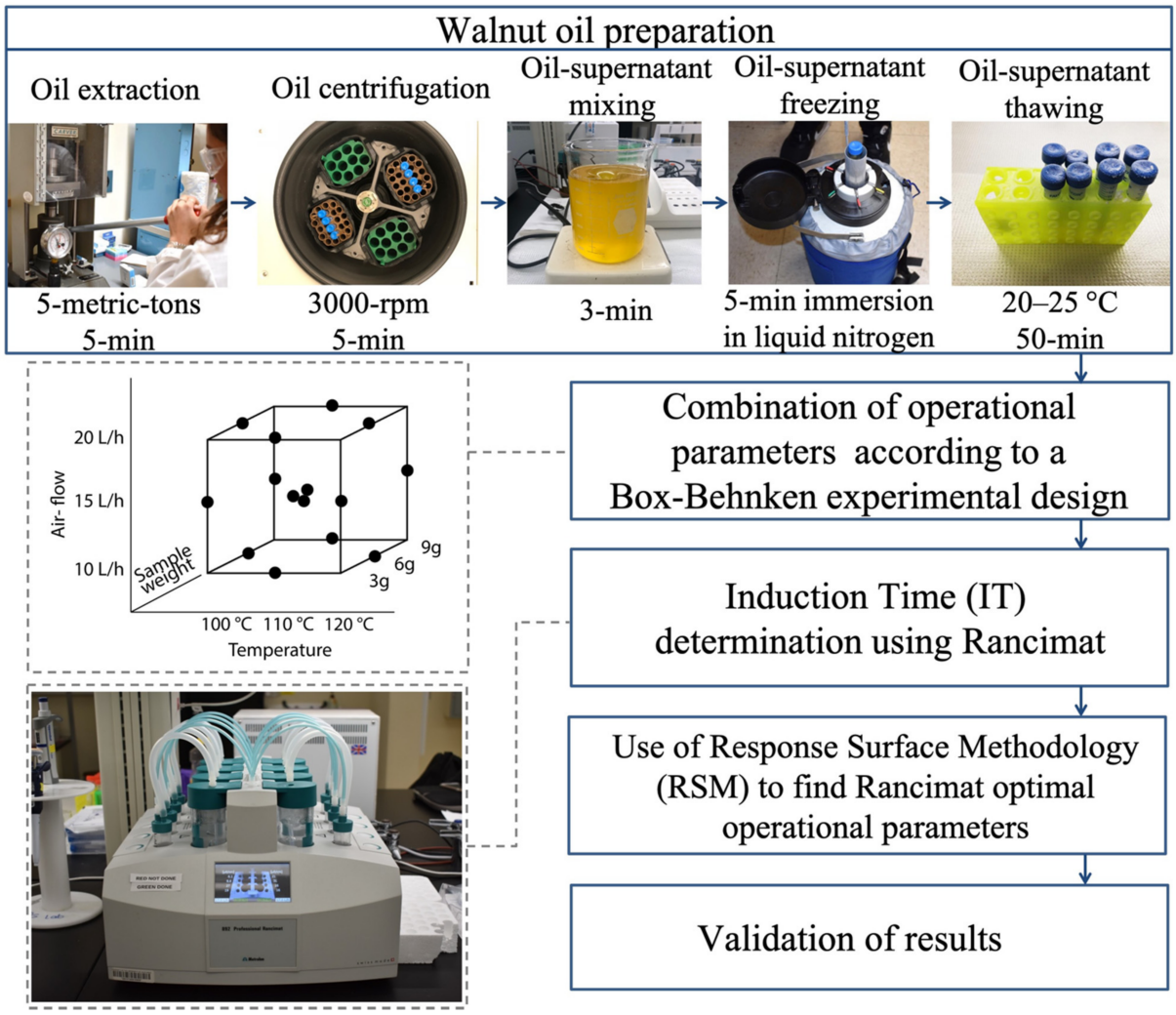

Figure 1. Description of the followed methodology to optimize Rancimat operational parameters.

For this study, oil extraction was repeated for approximately 70 batches, producing a total of 1 L of oil. The extracted oil was divided into 50 mL centrifuge tubes, and centrifuged at 3000 rpm for 5 min to precipitate the solid particles from the supernatant. Oil supernatant was collected to avoid the potential influence of solid particles on *IT* estimation. To ensure uniformity, the oil was poured in a 2 L glass beaker and stirred for 3 min on a stirring plate. Thereafter, the walnut oil was divided into 15 mL polypropylene vials and immediately immersed in liquid nitrogen for 5 min and stored at −80 °C for further analysis.

2.2. Optimization of Rancimat Operational Parameters (Independent Variables) Using Response Surface Methodology (RSM) and Box-Behnken Experimental Design

RSM using a three factor, three level Box–Behnken statistical design was used to optimize the Rancimat operational parameters (sample weight, temperature, and airflow) by maximizing the CV^{-1} [14]. In parallel, a model for the effect of operational parameters on the *IT* was also generated. The latter was performed to offer an in-depth understanding of the effect of optimized operational parameters in the walnut oil *IT*, and their implementation feasibility. R (V1.3.1093, R Development, Core Team, Vienna, Austria) software was

used to develop the models. The models with coded-factors for the experimental design is described in Equation (1).

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where the index n in Y refers to each response (dependent) variable ($Y_1 = CV^{-1}$ and $Y_2 = IT$); b_0 is the regression intercept; b_1 through b_3 are the regression coefficients; and X_1, X_2, and X_3 are the Rancimat operational parameters (sample weight, temperature, and airflow) or independent variables [12].

Levels for each operational parameter were coded as low (−1), medium (0), and high (+1), as described in Myers, Montgomery [12] (Table 1a). Table 1b shows the combinations of operational parameters on each run of the Box–Behnken statistical design.

Table 1. (**a**) Box–Behnken statistical design factors. (**b**) Combination of factors in Box–Behnken statistical design.

(**a**)			
	Levels (coded)/uncoded		
Operational parameters (units)	Low (−1)	Medium (0)	High (+1)
Sample weight (g)	3	6	9
Temperature (°C)	100	110	120
Airflow ($L \cdot h^{-1}$)	10	15	20

(**b**)			
	Block 1		
	Independent variables (operational parameters)		
Run	Sample weight (g)	Temperature (°C)	Airflow ($L \cdot h^{-1}$)
1	3	110	10
2	3	100	15
3	3	120	15
4	3	110	20
5	6	100	10
6	6	120	10
7	6	110	15
8	6	110	15
9	6	110	15
10	6	100	20
11	6	120	20
12	9	110	10
13	9	100	15
14	9	120	15
15	9	110	20
	Block 2		
	Independent variables (operational parameters)		
Run	Sample weight (g)	Temperature (°C)	Airflow ($L \cdot h^{-1}$)
16	3	100	10
17	9	120	20

2.3. Walnut Oil IT and CV (RSM Models Dependent Variables)

Walnut oil *IT* was evaluated using a Metrohm Rancimat Model 892 (Metrohm Ltd., Herisau, Switzerland). Eight vials containing walnut oil were removed at a time from storage (−80 °C), as this is the maximum number of samples that Rancimat can analyze at the same time. Before analysis, closed vials were thawed at room temperature (between 20 to 25 °C) for approximately 50 min. According to the Box–Behnken experimental design (Table 1a,b), walnut oil equivalent to 3, 6, or 9 g was added to each reaction vessel, and heated to 100, 110, or 120 °C, while 10, 15, or 20 $L \cdot h^{-1}$ of filtered air was forced through the oil within the reaction vessels. Each operational parameter combination (run) was

carried out in triplicate (n). Glass and plastic vessels were used and cleaned as described by the Rancimat manufacturer Metrohm [17]. The *IT CV* was calculated per run, as specified in Farhoosh [18], using Equation (2).

$$V = \frac{\sqrt{\sum_{i=1}^{n}\left(\left|IT - \frac{\sum_{i=1}^{n} IT}{n}\right|\right)^2}}{\frac{\sum_{i=1}^{n} IT}{n}} \quad (2)$$

where CV is the Coefficient-Of-Variation of the IT measurements per run (Table 1b), and n is the number of replications per run (3).

Values for the CV were expected to be near zero, as the oil samples and corresponding replicates were obtained from the same batch of oil and were exposed to the same handling and storage conditions. To facilitate data visualization and statistical analysis, the response variable (CV) was inversed (CV^{-1}).

2.4. Validation of Optimal Rancimat Operational Parameters

To validate the RSM model optimization results, an independent test was ran, in duplicate, using the optimized operational parameters (sample weight, temperature, and airflow) that correspond to the RSM stationary point as the CV^{-1} was maximized (validations-1). In addition, an independent test was run, in duplicate, after the effect of the optimized parameters onto the IT were considered (validations-2).

The same walnut oil was used to optimize and perform model validation-1 and -2. Ultimately, a paired t-test at 95% confidence was performed to evaluate if there was a significant difference in the CV and IT between validation-1 and -2. R (V1.3.1093, R Development, Core Team, Vienna, Austria) software was used to perform the t-test.

3. Results and Discussion

3.1. Optimization of Rancimat Operational Parameters (Independent Variables)

As shown in Table 2, the results for the lack of fit test were non-significant (p-value > 0.05), suggesting that for each model (RSM for CV^{-1} and IT), the second order non-linear models adequately described the relationship between the operational parameters (sample weight, temperature, and airflow) and the response variables (IT and CV^{-1}) [12]. This is an indicator that the RSM stationary points are relevant and models were properly developed [19].

Table 2. Regression coefficients and p-value [a] for each non-linear response variable model.

Term (Code)	CV^{-1}		IT (h)	
	Coeff.	***p*-Value**	**Coeff.**	***p*-Value**
Intercept (b_0)	1.1908	**0.0110 a**	2.7166	**4.137×10^{-10} a**
Block effect	0.8066	0.2939	0.0255	0.7557
Sample weight (X_1)	−0.0306	0.8746	0.0165	0.4565
Temperature (X_2)	−0.2111	0.2999	−1.9661	**9.570×10^{-11} a**
Airflow (X_3)	0.1911	0.3442	0.0362	0.1330
Temperature x sample weight (X_2X_1)	−0.0337	0.9093	−0.0025	0.9400
Airflow x sample weight (X_3X_1)	−0.2764	0.3685	0.0117	0.7248
Temperature x airflow (X_2X_3)	−0.3699	0.2410	−0.0317	0.3572
Sample weight (X_1^2)	−0.4239	0.2020	0.0086	0.8025
Temperature (X_2^2)	−0.2150	0.4948	0.6221	**1.475×10^{-6} a**
Airflow (X_3^2)	−0.2759	0.3872	0.0279	0.4319

[a] ANOVA for each term at $p = 0.05$. Statistically significant values (p-value ≤ 0.05) are in bold text.

The regression coefficients for the CV^{-1} optimization model (Table 2) suggested that none of the operational parameters (sample weight, temperature, and airflow) or interaction of these significantly contributed to the model, as the p-values for all terms were significant (p-values ≤ 0.05). The mathematical relationship between the response (dependent variable) and operational parameters can be understood by interpreting the regression coefficients

of the polynomial equation (Table 2) [14]. The coefficients represent the expected change in the response per unit change in each operational parameter (independent variable), when the remaining operational parameters are held constant [12].

Table 3 displays the results of ANOVA for the CV^{-1} model, where an R^2 of 57.8% and an R^2_{adj} of −12.4% were obtained. According to Myers, Montgomery [12], when R and R^2_{adj} differ dramatically, there is a high probability that non-significant terms have been included into the model [12]. The fact that none of the regression coefficients were significant could explain why R^2 and R^2_{adj} values differ substantially. The previous also indicates that the *CV* is highly variable within the range of evaluated parameters. Table 3 shows that for the CV^{-1} model none of the linear, quadratic, and polynomial order terms were significant as the three terms yielded a *p*-values > 0.05. This confirms that none of the operational parameters significantly contributed to the CV^{-1} model, and reiterates that the Rancimat equipment performs with high accuracy and consistency, regardless of operational parameters setup [12]. Nevertheless, a CV^{-1} maximum stationary point was discerned, as an indicator of low variation within a specific range of operation parameters, as three negative eigenvalues were obtained (Table 4) [12]. The optimized response (lowest variation) for CV^{-1} was established at 3.9 g for sample weight, 85.5 °C for temperature, and 26.7 $L{\cdot}h^{-1}$ for airflow. The stationary point for sample weight was inside the experimental region, while the stationary points for temperature and airflow were outside the experimental region. In RSM, it is possible to find a stationary point that is beyond the experimental region. This is called a rising or falling ridge. Therefore, if possible, further experiments need to be explored following the ridge path.

Table 3. ANOVA table for the inverse of coefficient of variation (CV^{-1}), induction time (*IT*), and non-linear response models.

Term	*df*	*SS*		*MS*		*F*-Value		*p*-Value [a]	
		CV^{-1}	*IT*	CV^{-1}	*IT*	CV^{-1}	*IT*	CV^{-1}	*IT*
Block	1	0.159	0.170	0.159	0.170	0.492	41.950	0.509	**0.0064**
Linear	3	0.661	38.261	0.220	12.753	0.681	3144.644	0.594	**5.616×10^{-10} [a]**
Quadratic	3	0.857	0.005	0.285	0.0015	0.883	0.378	0.500	0.772
Polynomial	3	0.985	1.436	0.328	0.4787	1.015	118.029	0.449	**1.006×10^{-5} [a]**
Residuals	6	0.941	0.024	0.323	0.0041				
Lack of fit	4	0.865	0.018	0.216	0.0044	0.402	1.293	0.801	0.478
Pure error	2	1.075	0.007	0.537	0.0034				

[a] ANOVA for each term at *p*-value = 0.05. Statistically significant values (*p*-value ≤ 0.05) are in bold text.

Table 4. Stationary maximized points in original units for each non-linear response variable model.

Response Variable	*Operational Parameters*			*Eigenvalues*		
	Sample Weight (g)	Temperature (°C)	Airflow ($L{\cdot}h^{-1}$)	Sample Weight (g)	Temperature (°C)	Airflow ($L{\cdot}h^{-1}$)
CV^{-1}	3.9	85.5	26.7	−0.550	−0.041	−0.323
IT	2.8	125.9	17.3	0.006	0.622	0.029
Mean	3.3	105.7	22.0			
Max	3.9	125.9	26.7			
Min	2.8	85.5	17.3			
Optimized	3.9	100	25			

The surface regression plots in Figure 2a–c show that at a lower temperature and higher airflow, the CV^{-1} increases, therefore decreasing the *CV*. This indicates that the conditions under which walnut oil oxidizes are more variable as temperature increases and airflow decreases [18]. A rising ridge in the direction of a lower temperature and a higher airflow can also be observed, indicating further analysis must be performed on those directions [12,19]. However, the maximum airflow set point for the Rancimat equipment is 25 $L{\cdot}h^{-1}$ and it is impossible to perform studies above that value. Regardless, this study demonstrated that at the optimized stationary point for a high sample weight

(3.9 g), low temperature (85.5 °C), and high airflow (26.7 L·h^{-1}) air has the potential to evenly saturate the walnut oil, yielding a less variable *IT* [18]. This result agrees with the reported by Farhoosh [18], and Jebe and Matlock [20] that concluded that a reduction in temperature leads to a decrease in the *IT CV*.

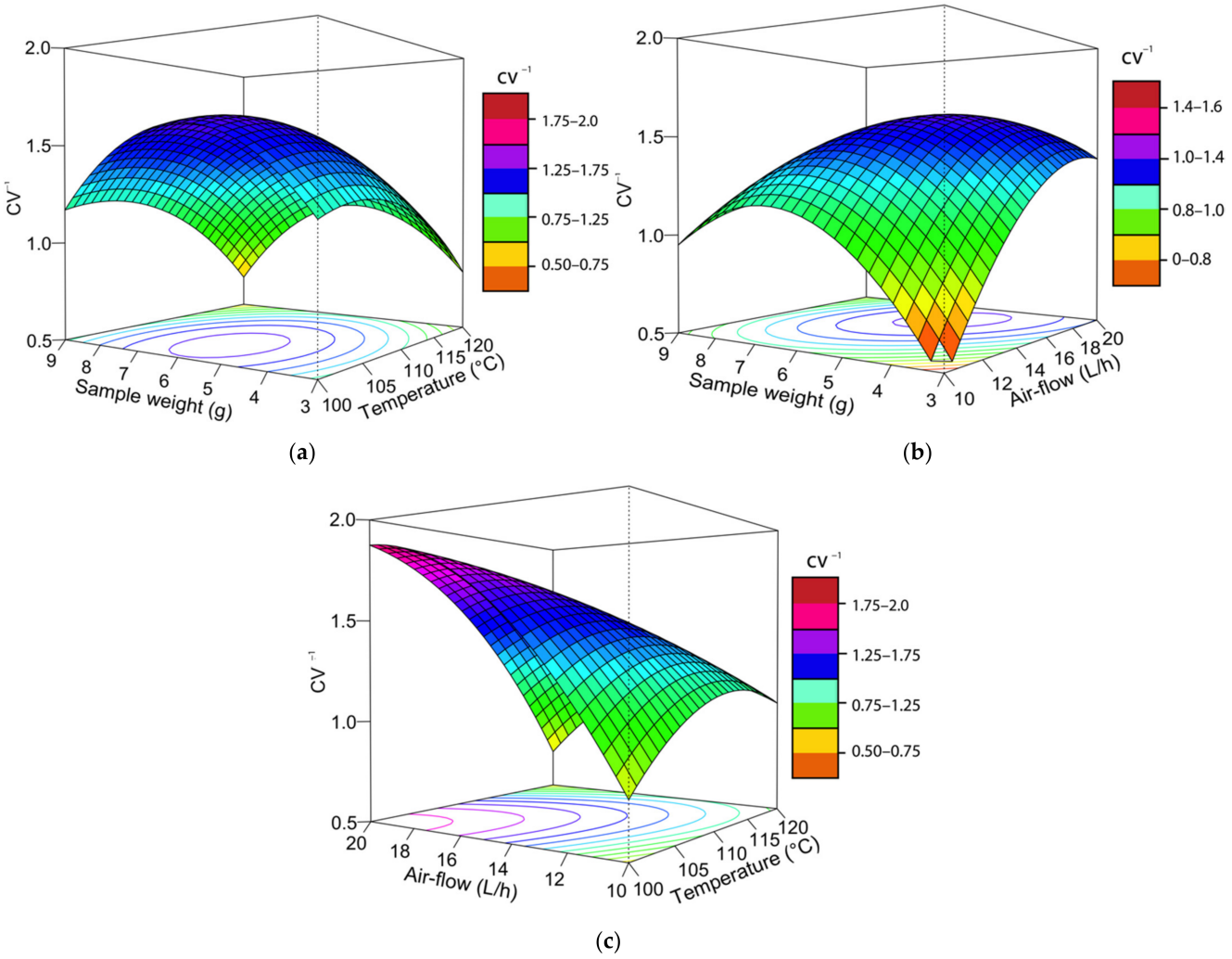

Figure 2. Surface and contour plots for the inverse of the coefficient of variation (CV^{-1}) versus (**a**) sample weight (g) and temperature (°C); (**b**) sample weight (g) and airflow (L·h^{-1}); and (**c**) airflow (L·h^{-1}) and temperature (°C).

If optimized conditions to minimize the *CV* are considered, it can be predicted that the *IT* will equal 21.31 ± 0.80 h. The previous was concluded, based on the generated *IT* model, as the *IT* at the lowest evaluated temperature (100 °C) equaled 5.33 ± 0.20 h. *IT* at the optimized conditions, which reflects the time of each test, is high and not practical for researcher and industrial application, especially as many samples need to be evaluated at once. In addition; even though a stationary point for the CV^{-1} optimized model was resolved, because none of the operational parameters (sample weight, temperature, and airflow) or interaction of these significantly contributed to the model, it is required to further explore the effect of operation parameters on the *IT* model.

Regarding the *IT* model, the *p*-value for the temperature regression coefficient was <0.05, suggesting that the effect of temperature in the *IT* was statistically significant. Sample weight, and airflow appeared to be non-significant, and no interaction of parameters was significant (*p*-value > 0.05). The temperature regression coefficient yielded a negative value, indicating that temperature has an antagonistic effect in the response, or the *IT* significantly decreases at a higher temperature. The *IT* response surface plots (Figure 3a–c)

visually confirm that temperature was the only variable that significantly affected the *IT*, and that the *IT* decreases with an increase in temperature. This behavior was expected, as it is known that the rate of an oxidation reaction is exponentially related to temperature [9,21]. These results coincided with those obtained by [10,18,21], who found a similar relationship between the *IT* and temperature.

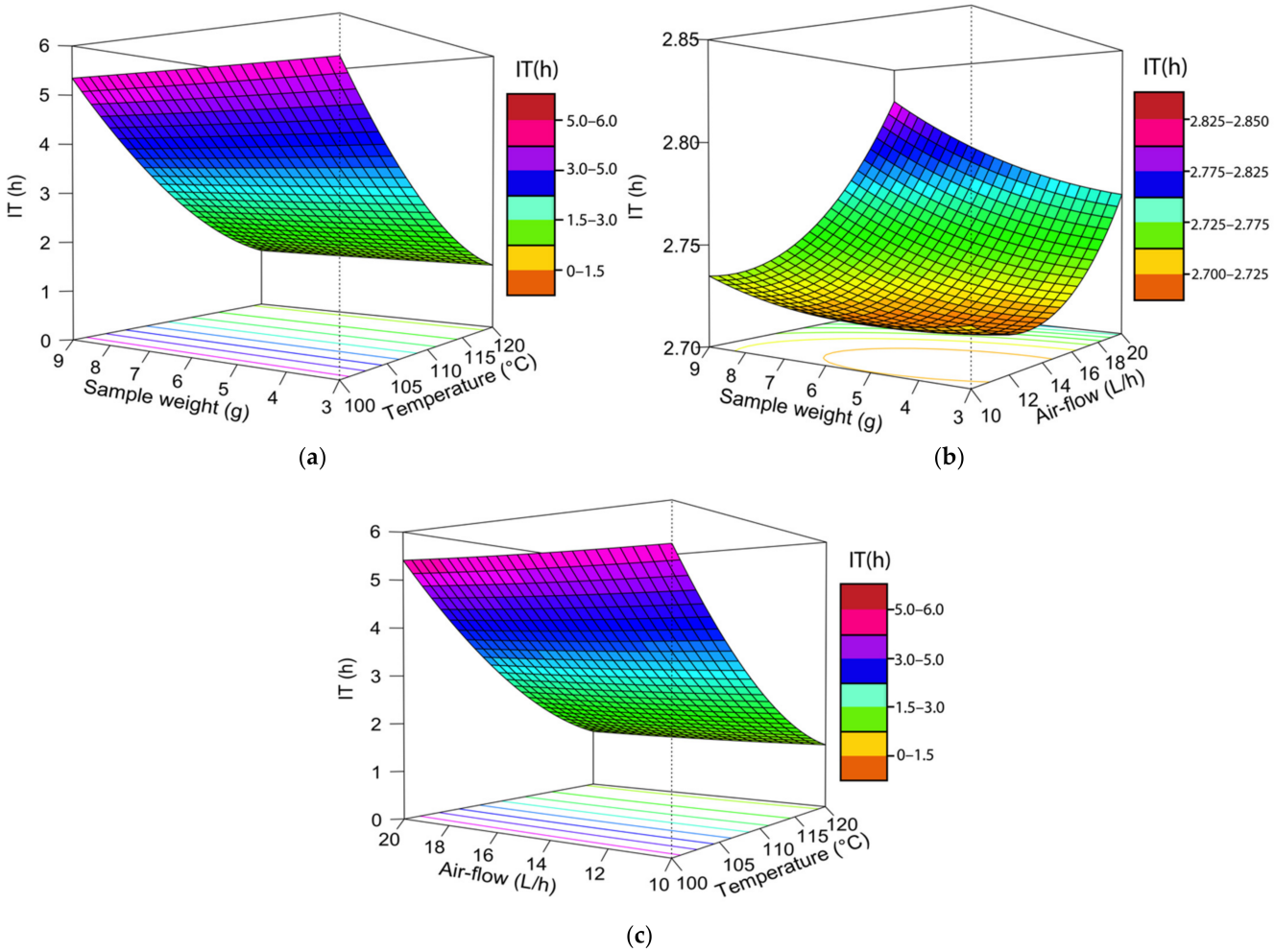

Figure 3. Surface and contour plots for the induction time (*IT*-h) versus (**a**) sample weight (g) and temperature (°C), (**b**) sample weight (g) and airflow ($L \cdot h^{-1}$), and (**c**) air-flow and temperature.

The results of the ANOVA for *IT* are shown in Table 3. It was determined that the R^2 and R^2_{adj} (adjusted for degrees of freedom) were 99.9% and 99.8%, respectively. This means that the operational parameters (sample weight, temperature, and airflow) significantly explained the variation in the *IT* [14]. Moreover, the *p*-value for the first and polynomial order terms were <0.05. Therefore, the hypothesis that none of the parameters significantly contribute to the model was rejected [12]. In other words, the canonical analysis or relationship between the response and operational parameters was relevant [12,14].

For the *IT* model, a ridge is present in the sample weight equal to 2.8 g, and a temperature of 125.9 °C, advising that sample sizes below 2.8 g, and temperatures above 125.9 °C should be explored. The Rancimat upper achievable temperature limit is 120 °C; therefore, it is not possible to perform an experiment at temperatures above 125.9 °C. Moreover, previous studies have determined that temperature stabilization is unreliable at a sample below 2.5 g. It is hypothesized that air-saturation cannot be maintained for a low sample weight, when exposed to high airflow rates [10,20]. Figure 3b shows that the *IT* increases from 2.72 to 2.82 h, as the sample weight and airflow increase to their maximum

values. A potential reason is that as the sample weight increases, the airflow becomes limited, making it impossible to maintain the air-saturated conditions, as required to uniformly induce the oil oxidation [10]. For these reasons, it was concluded that exploring additional conditions for the *IT* model was not feasible and will not lead to a significant improvement. Hence, if only the *IT* model was to be considered, the *IT* would be at its minimum when a sample weight, temperature, and airflow equaled 2.8 g, 125.9 °C, and 17.4 L·h^{-1}, respectively.

Studying and analyzing the relationship between operation parameters on both response variables (CV^{-1} and *IT*) was crucial to properly optimize the Rancimat method to accurately and feasible evaluate walnut oil *IT*. Optimized operational parameters need to consider the Rancimat equipment capabilities, and analysis time, while minimizing the *IT* CV (model that maximizes the CV^{-1}). Therefore, final optimized parameters for sample weight, temperature, and airflow were established at 3.9 g, 100 °C, and 25 L·h^{-1}, respectively (Table 4). Table 4, shows that the final optimized parameters are close to the arithmetic mean of operational parameters that minimize the *IT*, which ultimately defines the sample analysis time, and maximize the CV^{-1} (sample weight = 3.3 g, temperature = 105 °C, and airflow = 22 L·h^{-1}). As it was determined through the *IT* RSM model that the sample weight and airflow do not have a significant effect on the *IT*, values for these two parameters were selected whenever they minimize the CV, while falling within the experimental region and the Rancimat equipment capabilities.

3.2. Validation of optimal Rancimat Operational Parameters

As shown in Table 5, a CV of 0.966 ± 0.851%, and an *IT* of 16.261 ± 0.360 h were observed, when applying the operational parameters that exclusively maximized the CV^{-1} (validation-1). On the other hand, the final operational parameters yielded a CV and an *IT* equal to 1.259 ± 0.838 and 5.421 ± 0.016, respectively. The CV obtained in validation-1 and validation-2 were not significantly different (p-value > 0.05, df = 1.99, t-statistic = −0.34), while the *IT* was significantly different (p-value ≤ 0.05, df = 1.00, t-statistic = 42.46). These results are illustrated in Figure 4. The *IT* from validation-2, is approximately a third of the *IT* from validation-1, without significantly increasing the results variability (CV). A lower *IT*, as observed in the final operational parameters, represents an advantage as a significantly larger number of samples can be evaluated within the same timeframe.

Table 5. Mean and standard deviation of the *IT* (h), CV (%), and CV^{-1} obtained from the validation of operational parameters at the stationary point (Validation-1) and optimal parameters (Validation-2).

Validation	Sample Weight (g)	Temperature (°C)	Air Flow (L·h^{-1})	*IT* (h)	CV (%)	CV^{-1}
Validation-1 (Validation at the maximized stationary point for CV^{-1})	3.9	85	25	16.261 ± 0.360	0.966 ± 0.851	1.688 ± 1.486
Validation-2 (Validation at final optimal parameters)	3.9	100	25	5.421 ± 0.016	1.259 ± 0.838	1.020 ± 0.679

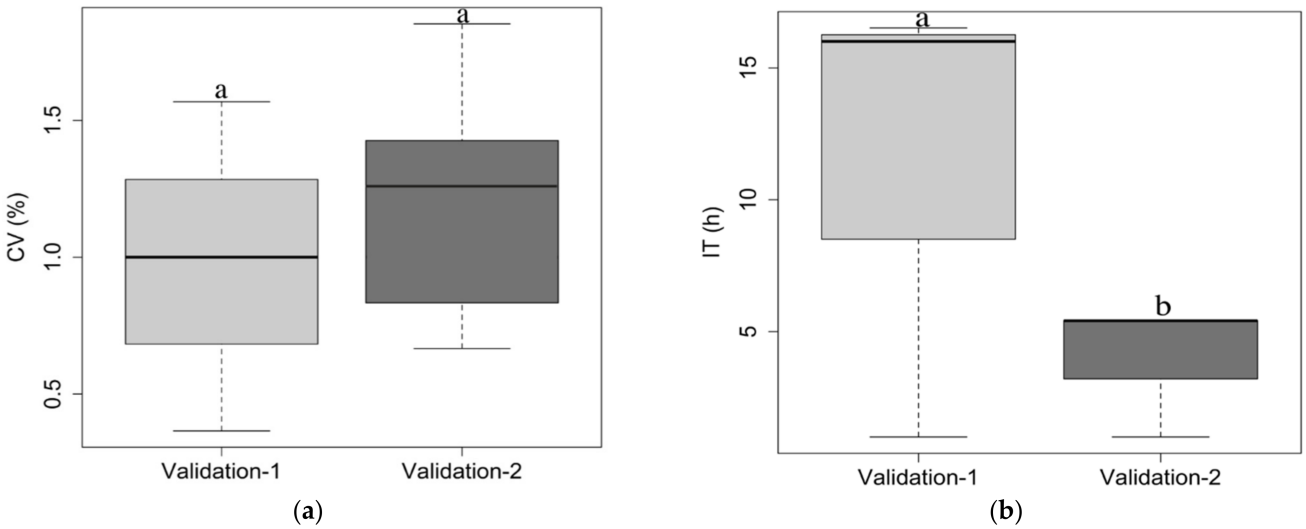

Figure 4. Box-plots showing: (**a**) The *CV* (%); and (**b**) *IT* (h) distributions in Validation-1 and Validation-2. The median is represented as a thick horizontal black line, upper and lower quartiles as a box with the maximum and minimum measurements as lines protruding from these. Box-plots followed by a different letter within each boxplot set are significantly different between each other (p-value ≤ 0.05).

4. Conclusions

Using the second-order RSM prediction models for each individual response variable (CV^{-1} and *IT*) yielded an optimized combination of Rancimat operational parameters for sample weight, temperature, and airflow equal to 3.9 g, 100 °C, and 25 L-h^{-1}, respectively. Final operational parameters yielded a *CV* of 1.259 ± 0.838%, and *IT* of 5.421 ± 0.016 h. RSM proved to be a useful methodology to properly optimize the Rancimat operational parameters (sample weight, temperature, and air flow) that allow us to precisely, accurately, and efficiently determine walnut oil *IT*. Choosing the appropriate temperature level is pivotal in determining walnut oil *IT* using the Rancimat equipment, while the airflow rate and sample size proved to not have a significant effect.

Even though the Rancimat equipment evaluates the relative oxidative stability of walnut oil, the application of the optimized operational parameters, as a standard method for future walnut oil *IT* determination, can lead to comparable results between studies. In addition, having access to a reliable and accurate method of determining walnut rancidity will facilitate the application of alternative postharvest and food processing techniques to improve walnut quality, lipid stability, and nutritional value.

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