Experimental design for ultrasound-assisted extraction of *Schinus terebinthifolius*

Planejamento experimental para a extração assistida por ultrassom de *Schinus terebinthifolius*

Diseño experimental para extracción asistida por ultrasonido de *Schinus terebinthifolius*

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Abstract
The need for efficient extraction of plant constituents with a high yield and purity and limitations to conventional extraction techniques that are limited by mass transfer, has resulted in the development of new extraction processes. Sonochemistry involves cavitation induced by pressure fluctuations generated by ultrasound waves in a liquid medium. Ultrasound can be effectively used to increase the yield and the mass transfer rate in solid-liquid extraction processes. The present work employed a factorial design 33 and a central composite design (PCC) for the extraction of *Schinus Terebinthifolius*. It is possible to verify an optimum point for the extraction (35.24%) for a mass of 13.49 g and an extraction time of 2 h, at 60 ºC.
Keywords: Extraction; Ultrasound; *Schinus terebinthifolius*; Experimental design.

Resumo
A necessidade de extração eficaz de plantas com alto rendimento e pureza, além do fato das técnicas convencionais de extração serem limitadas pela resistência de transferência de massa resultou no desenvolvimento de novos processos de extração. A Sonoquímica, envolve a cavitação induzida pelas flutuações de pressão geradas pelas ondas de ultrassom em um meio líquido. O ultrassom pode ser eficazmente utilizado para aumentar o rendimento e a taxa de transferência de massa, em processos de extração sólido-líquido. O presente trabalho empregou um planejamento fatorial 3^3 e um planejamento composto central (PCC) para a extração de *Schinus terebinthifolius*. Sendo possível verificar um ponto ótimo para a extração (35,24%) com a massa de 13,49 g e tempo de extração de 2 h, à 60 ºC.
Palavras-chave: Extração; Ultrassom; *Schinus terebinthifolius*; Planejamento experimental.

Resumen
La necesidad de una extracción efectiva de plantas con alto rendimiento y pureza, además del hecho de que las técnicas de extracción convencionales están limitadas por la resistencia a la transferencia de masa, ha resultado en el desarrollo de nuevos procesos de extracción. La ecoquímica implica la cavitación inducida por las fluctuaciones de presión generadas por las ondas de ultrasonido en un medio líquido. El ultrasonido se puede usar efectivamente para aumentar el rendimiento y la tasa de transferencia de masa, en los procesos de extracción sólido-líquido. El presente trabajo empleó un diseño factorial 33 y un diseño compuesto central (PCC) para la extracción de *Schinus terebinthifolius*. Es posible verificar un punto óptimo para la extracción (35.24%) con una masa de 13.49 gy un tiempo de extracción de 2 h, a 60 ºC.

Palabras clave: Extracción; Ultrasonido; *Schinus terebinthifolius*; Diseño experimental.

1. Introduction

Solid-liquid extraction is used in many biotechnological and pharmaceutical industrial processes. Plant materials contain only a small amount of active solute, but in most cases the high value of these compounds justifies the development of high-performance separation processes. The need for effective extraction of biologically active components from plants, without any loss of activity and high purity, has resulted in the development of new extraction processes (Chemat et al., 2016; Vernès, Vian & Chemat, 2020).

Conventionally, different mechanical and chemical processes, such as solvent extraction, steam distillation, high hydrostatic pressure extraction, pulsed electric field processes, high pressure processes, amongst others, are used for the extraction of products from plant materials. The selection of the method to extract active components with maximum yield and a high degree of purity, depends mainly on the nature of the compounds, the thermal stability and the nature of the raw materials to be processed (Vernès, Vian & Chemat, 2020).

Conventional extraction techniques are often limited by mass transfer resistance, due to the involvement of more than one phase in the system. These methods are time-consuming and may require extended operation times, depending on solvent diffusion rates. High energy consumption is also a disadvantage of conventional extraction processes. In the case of extraction of sensitive active compounds, the active molecules can be destroyed due to the use of pressure and severe temperature conditions (Shirsath, Sonawane & Gogate, 2012; Chemat et al., 2016).

Problems related to conventional techniques have motivated the investigation of new techniques that are, at least, equally efficient and, at the same time, economically viable. Recently, the application of ultrasound techniques in solvent extraction has attracted more attention. The use of ultrasound results in "cavitation", which is described as the formation, growth, and collapse of gas-filled bubbles in a liquid. Sonochemistry specifically involves cavitation induced by pressure fluctuations generated by ultrasound waves in a liquid medium. Cavitation generated using ultrasound is known to produce physical effects such as liquid circulation currents and turbulence that can lead to a significant increase in mass transfer rates. Ultrasound can be effectively used to increase the yield and the mass transfer rate, in several solid-liquid extraction processes (Goula, Ververi, Adamopoulou & Kaderides, 2017).

The mechanism of intensification of extraction efficiency using ultrasound has been attributed to the propagation of ultrasound pressure waves through solvents, resulting in the phenomenon of cavitation. The ultrasound-assisted extraction control mechanism is generally attributed to mechanics, cavitation, and the thermal effects that can result in cell wall disruption, particle size reduction, and improved mass transfer across cell membranes. The implosion of cavitation bubbles generates microturbulence, high speed collisions between particles and disturbance in micro-porous particles of biomass, which accelerate diffusion. Due to the limited space for bubbles to expand, most bubbles collapse asymmetrically, resulting in liquid circulation currents with intense turbulence. In addition, cavitation on the material surface results in surface erosion and particle breakdown. Thus, it appears that the application of ultrasound allows the target compounds to dissolve in the solvent thus increasing the yield at lower times by disturbing the cell wall (Shirsath, Sonawane & Gogate, 2012; Goula, Ververi, Adamopoulou & Kaderides, 2017; Vernès, Vian & Chemat, 2020).

Due to cavitation, cracks develop in the cell wall which increase the permeability of plant tissues and this facilitates the entry of the solvent into the material. In addition, turbulence and acoustic transmission can significantly increase the solid-liquid COEF mass transfer coefficient due to effects on the microscale system. The mechanical effects of ultrasound can also increase the contact surface between the solid and liquid phases, due to the possibility of reducing the size of the solid matrix. The improved rates of mass transfer occur due to the increased speed with which the solvent reaches the solid surface and also

the transfer of components soluble in solvents will be higher (Vernès, Vian & Chemat, 2020).

Essential oils are usually extracted by distillation or solvent extraction. The oils are used in perfumes, cosmetics, soap and other household cleaning products, to flavour foods and drinks and in medical applications. These essential oils can be extracted from the residues of raw materials or natural products derived from plants and the efficiency of the extraction technique decides the cost of the essential oils obtained. For the removal of lipophilic compounds from the interior of plant tissues, classic extraction technologies are based on the use of an appropriate solvent. The choice of a suitable solvent, in combination with sufficient mechanical stirring influences the mass transport processes and, as a result, the extraction efficiency. The most widely used solvent for extracting essential oils from plant sources is hexane. However, recently, the use of alternative solvents, such as alcohols (ethanol or isopropanol) has increased due to environmental, health and safety issues (Shirsath, Sonawane & Gogate, 2012).

According to Lorenzi (2009), *S. terebinthifolius* is the fruit of the tree popularly known as Aroeira-parsley belonging to the Anacardiaceae Family, and is also known as aroeirinha, aroeira-mansa, aroeira-falsa, aroeira-chorão, chorão, aroeira-periquita, anacauíta, aroeira-mole, aroeira-periquita, corneíba, pepper-of-turkey, aroeira-de-sabiá. It is a plant native to Brazil, but can be found in much of South America, where it has adapted to different climates. It is resistant to dry climates, frosts and droughts. It is widely used in urban landscaping. (Lorenzi, 2009).

S. terebinthifolius has commercial value for the production of cosmetics and drugs, and several parts of the plant can be used. The ripe fruit is slightly spicy and sweet and can be used as a condiment as can the dry and ground fruit, which resembles black pepper. In general it has as chemical constituents: resins, steroidal saponins, tannins, flavonoids, alkaloids, steroids, monoterpenes, triterpenes and sesquiterpenes, the latter three being most found in fruits (Bendaoud, Romdhane, Souchard, Cazaux & Bouajila, 2010; Martins, Arantes, Candeias, Tinoco & Cruzmorais, 2014; Alves, 2020).

Thus, the objective of this study was to perform a 33 factorial design, and a Central Composite Design using the STATISTICA® program, in order to obtain an optimal condition for the extraction of S. terebinthifolius. The following variables were considered: the mass of the pepper, extraction time and extraction temperature.

2. Methodology

The present work was a laboratory research on a qualitative basis (Pereira et al., 2018).

The experimental design was performed in order to minimize the number of tests and find better effects (yield), a fractional 33 factorial design (3 levels and 3 variables) was used in order to evaluate the independent variables such as pepper mass (X1), extraction time (X2) and extraction temperature (X3). To start the experimental design, 27 tests were carried out, the levels of each factor are shown in Table 1. For the experiments, the apparatus used is described in Figure 1. In this apparatus, the jacketed reactor, containing the sample (*S. terebinthifolius*) and ethyl alcohol (95%) was immersed in an ultrasonic bath that was always used at the same power intensity. The jacketed reactor was connected to a thermostatic bath that kept the temperature inside the reactor constant and a mechanical stirrer that kept the solution in constant agitation. At the end of the experiments, the data obtained from the percentage yield of the extractions were fed into the STATISTICA program to obtain the figures/graphs for the discussion of the results.

Figure 1: Experimental apparatus scheme: A- Ultrasonic bath; B- Jacketed reactor; C- Mechanical shaker and D- Thermostatic bath.
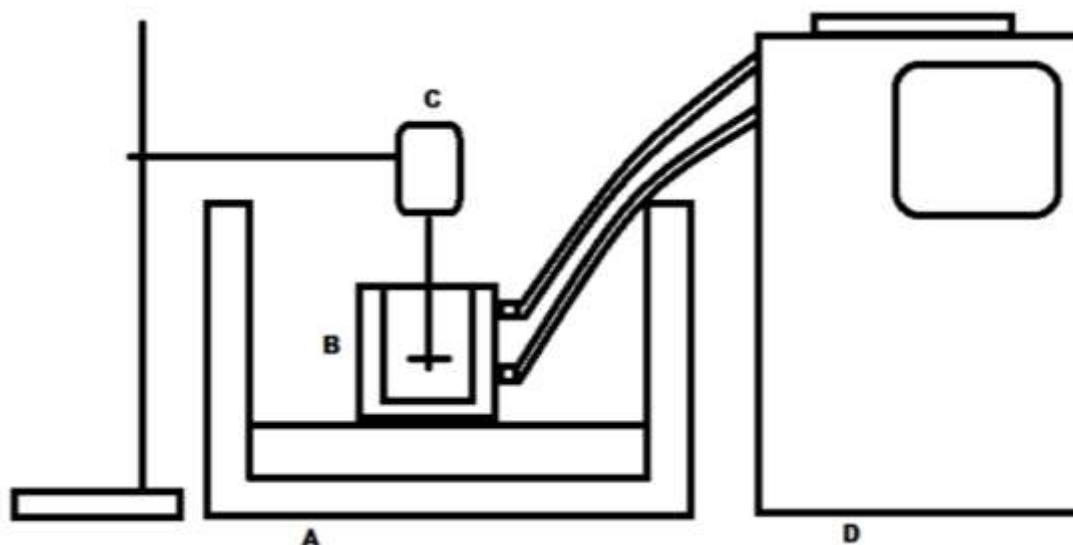
Source: Authors (2020).

Table 1: Factors with the coded (-1; 0; +1) and real values of fractional factorial design 3^3.

Variables	Levels		
	-1	**0**	**+1**
Pepper Mass (g)	20	40	60
Extraction time (h)	1	2	3
Extraction temperature (°C)	50	60	30

Source: Authors (2020).

It is noteworthy that ethyl alcohol was chosen as a solvent for this extraction because it has low vapor pressure, which results in the formation of a greater number of cavitation bubbles, whose collapse is intensified, producing high intensity shock waves, resulting in better mass transfer (Mandal, Dewanjee, Sahu & Subhash, 2009).

3. Results and Discussion

3.1 Experimental Design

3.1.1 Factorial Design

A factorial design (33) was performed to verify which variables were significant for the monitored response. The regression model was evaluated by analysis of variance (ANOVA) and f test with a 95% confidence interval. Response surfaces and Pareto graphs were used to visualize the relationship between the yield obtained and the experimental factors. The yield was obtained using **Equation 1**.

$$\text{Yield (\%)} = \frac{me}{ma} \cdot 100 \qquad \textbf{(1)}$$

In which: me: mass extracted; ma: mass of sample.

The experimental conditions and results obtained for mass, time and temperature are shown in Table 2.

Table 2: Experimental matrix for 33 factorial design.

Mass (g)	Time (h)	Temperature (°C)	Yield (%)
20	1	50	19.29
20	1	60	17.64
20	1	70	13.94
20	2	50	26.9
20	2	60	23.62
20	2	70	25.03
20	3	50	21.63
20	3	60	26.79
20	3	70	17.78
40	1	50	9.72
40	1	60	8.17
40	1	70	8.2
40	2	50	21.9
40	2	60	22.24
40	2	70	29.95
40	3	50	16.07
40	3	60	18.31
40	3	70	17.47
60	1	50	14.31
60	1	60	6.08
60	1	70	19.45
60	2	50	12.22
60	2	60	14.12
60	2	70	16.48
60	3	50	14.15
60	3	60	5.12
60	3	70	14.55

Source: Authors (2020).

The Pareto graph (Figure 2) indicates, in decreasing order of magnitude, the effects that were significant for obtaining greater yield. The vertical dashed line indicates the limit of the 95% significance level considered. The positive effects of the isolated variables indicate that the use of high levels is necessary to achieve the best response efficiency. In contrast, the negative effects indicate that lower levels can be used to achieve better results. From the respective values of the estimated effects, the mass of the pepper contributed negatively, and time had positive influence on the response of the process. According to Mandal et al. (2009), ultrasound-assisted extraction of curcumin, showed an increase of 31.4% in the extraction efficiency with an increase in the extraction time from 20 to 60 min, this is due to the greater exposure of the sample to shock waves to improve the balance of mass transfer. In addition, these shock waves can also cause some destructive changes in the plant's cell wall leading to leaching of constituents. Also, according to Mandal et al. (2009), ultrasound-assisted extraction can be effectively used to reduce plant extraction time and without the help of heat, as shown in the present study.

Figure 2: Pareto graph obtained by factorial design (33) as a function of yield (%).
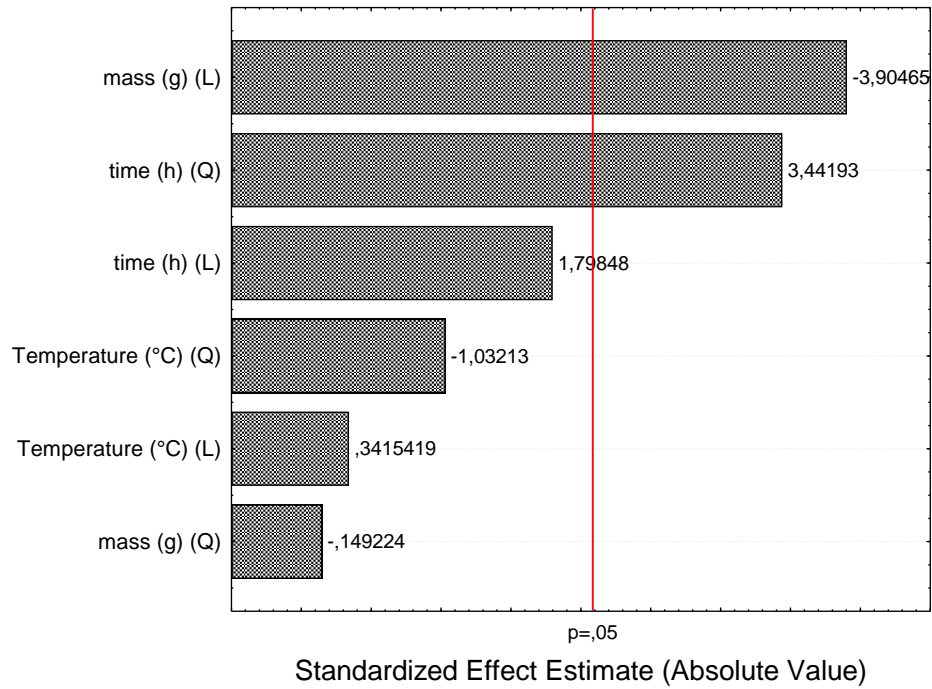
Source: Authors (2020).

The statistical significance of the main effects and their interactions were evaluated through analysis of variance (ANOVA), showed that the mass (linear term) and the extraction time (quadratic term) significantly affected the extraction of *S. terebinthifolius* ($p < 0.05$). The temperature and the interaction between the factors had no significant effects. The ANOVA results (Table 3) are in accordance with the data of the Pareto graphs (Figure 2), and the results of the factorial models predicted an R2 of 0.6119.

After processing the results, the estimation and validation of the mathematical model obtained is presented by **Equation 2**.

$$Y = 17,07889 - 8,46000X_1 + 6,45833X_2^2 \qquad \text{(2)}$$

3.1.2 Central Composite Design

After analyzing the significance of the three input variables and their respective levels, a central composite design (CCD) was carried out to verify the curvature of the plane. The statistical analysis of the experimental design allowed the verification that the temperature does not influence the process. Thus, to perform the PCC, an extraction at 60 ºC was adopted. As observed in 33 factorial design, the increase in extraction time and the reduction in mass increase the efficiency of the process. In view of this, the experimental matrix (Table 4) was generated, obtaining 11 experiments, carried out randomly, adopting a 95% confidence interval and shown in Table 5.

Table 3: Analysis of Variance (ANOVA).

	Sum of Squares	Degree of freedom	Mean of squares	F	p
mass (g) (L)	322.0720	1	322.0722	15.2463	0.0009
Mass (g) (Q)	0.4700	1	0.4704	0.022327	0.8829
time (h) (L)	68.3280	1	68.3281	3.2345	0.0872
time (h) (Q)	250.2600	1	250.2604	11.8469	0.0026
temperature (°C) (L)	2.4640	1	2.4642	0.1166	0.7363
Temperature (°C) (Q)	22.5040	1	22.5041	1.0653	0.3143
Error	422.4920	20	21.1246		
Total Sum of squares	1088.5910	26			

Source: Authors (2020).

Table 4: Levels for the central composite design for optimization of the experimental yield.

	Levels				
	-α	-1	0	+1	+α
Mass (g)	9.26	10	15	20	20.73
Time (h)	1.85	2	3	4	4.15

Source: Authors (2020).

The mathematical relationship between the response function and the independent variables can be approximated by a quadratic polynomial given by **Equation 3**, with $R^2 = 0.76151$.

$$Y = 32,37 - 7,12X_1 - 15,88X_1^2 - 6,57X_2 \qquad \textbf{(3)}$$

In which: Y: percentage extraction yield obtained; X_1: mass of sample used; X_2: time.

The coefficients of the quadratic model in Equation 3 were calculated by multiple linear least-squares regression analysis. Negative coefficients suggest that the reaction is favored in the presence of low levels of the respective variable within the studied range, while positive coefficients indicate that the yield is favored from a higher level. In Figure 3 (a), the response surface obtained is observed, and Figure 3 (b) the response outline. The graph of predicted values versus those observed (Figures 4) and the residuals graph (Figure 5), were constructed to analyses the fit and evaluate the model trend. Note that the experimental points follow the proposed model relatively well and that they oscillate around zero without any tendency.

Table 5: Central composite design and experimental results as a function of extraction yield (%).
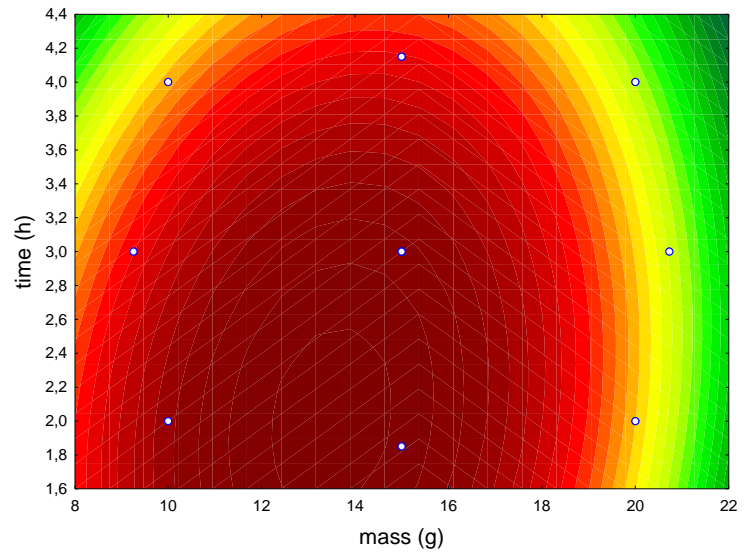
Mass (g)	Time (h)	Yield (%)
10	2	32.88
10	4	26.27
20	2	17.19
20	4	15.58
9.26	3	20.10
20.73	3	22.55
15	1.85	35.24
15	4.15	23.39
15	3	33.11
15	3	32.44

Source: Authors (2020).

Figure 3: Graphs of **(a)** response surface, and **(b)** contour as a function of Yield.
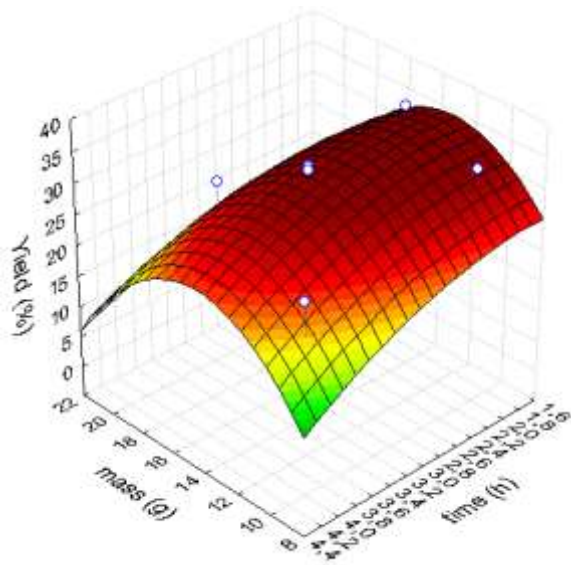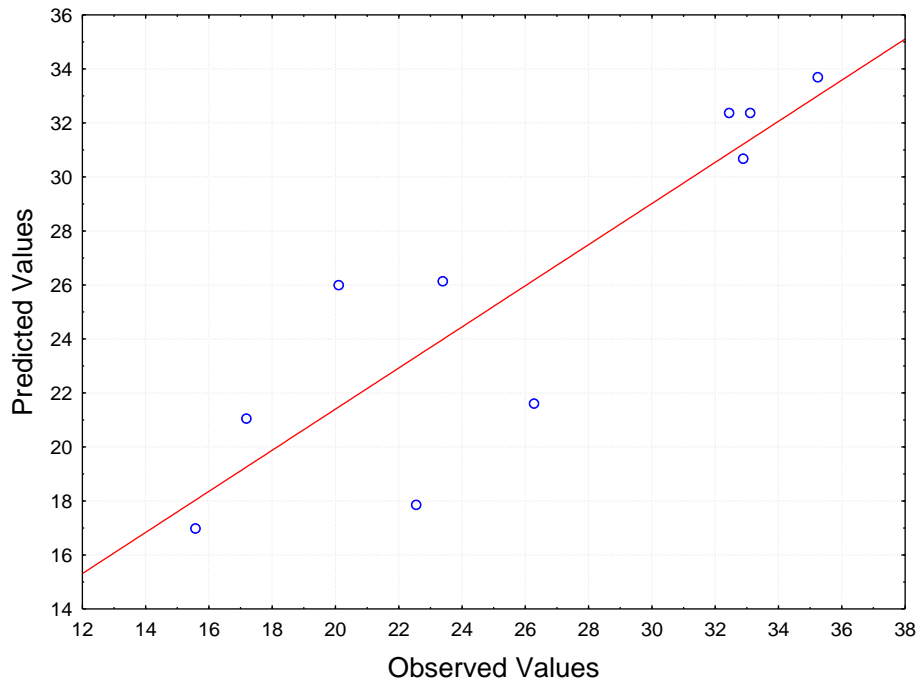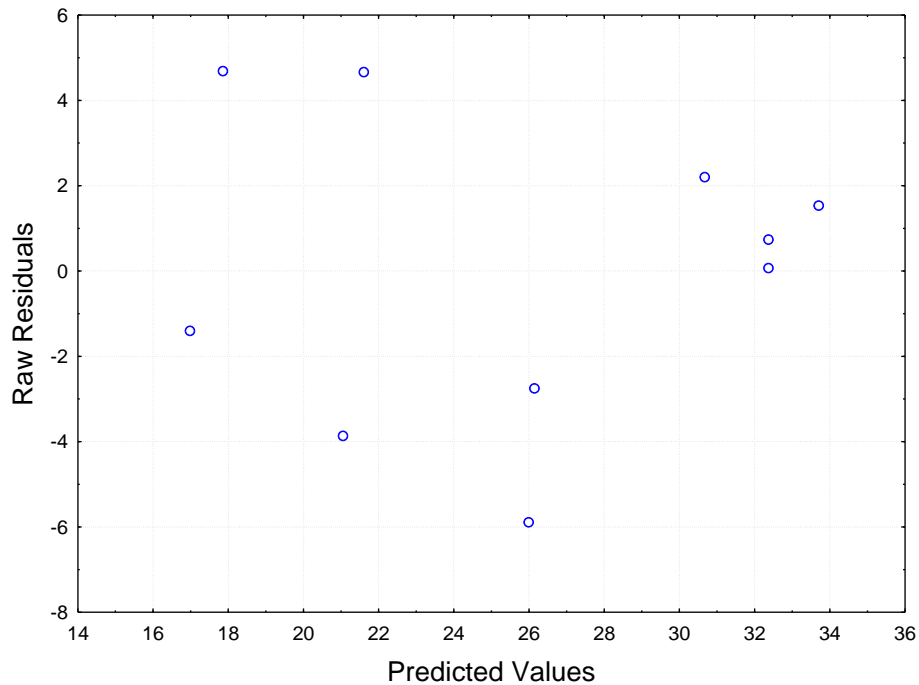

(a)

(b)

Source: Authors (2020).

Figure 4: Predicted versus observed values obtained by the central composite design.

Source: Authors (2020).

Figure 5: Graph of residues obtained by central composite design.

Source: Authors (2020).

The optimization of the process with PCC treatment, quantifies relevant factors and these contribute to the best parameter to be achieved. With this data, the optimized time and mass can reach a higher percentage of yield (Table 6). From this experimental matrix, within the critical values observed in Table 6, the predicted value is a yield of 34.53%, using 13.49g

mass and 2h of extraction. This value is significant, since, according to Oliveira Junior et al. (2013) *S. terebinthifolius* has between 5.50 and 8.81% of essential oil. Considering that in extraction using solvent and, in this specific case, ultrasound, in addition to the essential oil components other more polar compounds will also be extracted, the yield value obtained was very promising.

Table 6: Critical values for maximum production of extraction yield.

	Observed Minimum	Critical	Observed Maximum
Mass (g)	9.26	**13.49**	20.73
Time (h)	1.85	**2.0**	4.15

Source: Authors (2020).

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The purpose of experimental design is to detect the importance of the effects and this can be seen by the quadratic average or by the value of the effects. Thus, it appears that it is possible to observe significant values involving mass and time at the 95% confidence level, and the proposed model can explain about 76.14% of the variance (R^2) around the mean (Draft & Montgomery, 2003). In this way, the experimental model used proves to be important and significant.

4. Conclusion

Employing the 33 factorial design used, it was possible to find the optimal range for the extraction of *S. terebinthifolius*. Subsequently, a central composite design was performed to obtain the optimum point for the extraction. The optimal point of extraction according to the CCD was 13.49 g and 2 h of extraction. In general, ultrasound-assisted extraction of S. terebinthifolius proved to be feasible and more efficient than studies involving other extraction techniques.

In future studies, a mixture of water and ethanol will be employed because according to the increase in the polarity of the extraction solvent causes an effective swelling of the plant cell by water, which helps to increase the surface area of contact between the solute and the solvent. In addition, the presence of water decreases the viscosity of the mixture and, therefore, should improve mass transfer.

Acknowledgments

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