OPTIMIZATION OF EUGENOL PRODUCTION FROM CLOVES USING TAGUCHI DESIGN OF EXPERIMENTS

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***Abstract*— This work study the application of Taguchi method and factorial design of experiment for modeling and optimization of the influence of some variables in the extraction of essential oil of cloves by a lab scale microwave. This methodology was compared with conventional methods such as hydrodistillation, steam distillation, continuous solid-liquid extraction. Subsequently, the eugenol present in clove oil was isolated, purified and characterized by gas chromatography. Results showed that the yields obtained by microwave are highest respect to others methods being the optimum conditions to produce a 92.4 % of yield of eugenol a temperature of 70 °C, with a stirring of 480 rpm, in 25 minutes of process, using ethanol as solvent and a relation liquid-solid of 19 mL/mg.**

***Keywords*— Taguchi, Factorial Design, ANOVA Microwave-Assisted Extraction, Eugenol.**

I. INTRODUCTION

Essential oils are aromatic and volatile oil liquids extracted from different parts of plants (Guntero *et al.*, 2018), which usually consist of terpenes, terpenoids, alcohols, aldehydes, esters, ethers, ketones, phenols (Ali *et al.*, 2015). They are used in aromatherapy, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthesia (Bakkali *et al.*, 2008; Amorati *et al.*, 2013). Particularly, many phenylpropenes impart a characteristic aroma, and some fresh fruits and processed products that contain them are widely consumed (Koeduka *et al.*, 2013). Phenylpropanoids are a group of plant secondary metabolites and perform a vital role in plant communication, pollinator attraction and defense against herbivores and other pathogens (Anand *et al.*, 2016). Eugenol, a phenylpropene is found in bay leaves, allspice, and the oil of cloves that originate from the Syzygium species (Ito *et al.*, 2005). Has wide application in pharmaceuticals, cosmetics, dentistry, food, agriculture, pesticides (Shahavi *et al.*, 2015) and like a component of the packaging of aliment or cosmetic with antioxidant properties (Guntero *et al.*, 2019).

Clove (*Syzygium aromaticum*; synonym: *Eugenia cariophyllata*) is an important aromatic spice. Its essential oil contains mainly eugenol, and smaller amounts of eugenyl acetate and the terpenoid β- caryophyllene organic compounds (Ágilar-González and López-Malo, 2013). The traditional methods for the isolation and purification of chemical constituents from plants tissues require relatively long extraction time and big solvent amounts. Moreover, many natural products are thermally unstable and may degrade during thermal extraction and/or distillation (Kimbaris *et al.*, 2006), which requires knowing the best extraction conditions. In fact, process engineers want to determine the levels of the design parameters at which the response reaches its optimum. The optimum could be either a maximum or a minimum of a function of the design parameters (Aslan, 2007). On the other hand, is known that the microwave-assisted extraction (MAE) is one of the best green technologies with advantages like, high extraction efficiency, good reproducibility, low consumption of organic solvents and time and low carbon dioxide output. MAE is based upon the selective and rapid localized heating of moisture in the sample by microwaves. Due to the localized heating, pressure builds up within the cells of the sample, leading to a fast transfer of the compounds from the cells into the extracting solvent (Mandal and Mandal, 2010; Sides *et al.*, 2000). The matrix to be extracted (usually water-rich) is mixed with a solvent having a low dielectric constant, so that most of the heating effect will be concentrated on the plant material (Cravotto *et al.*, 2008).

Various extraction parameters that have influence upon the extraction process will depend on a number of steps controlling the transport of analytes from the matrix to the bulk fluid. Generally these parameters include extraction solvent composition, solvent volume, extraction temperature, extraction time, system pressures and the matrix characteristics (including pH, water content and total organic matter content) (Wang *et al.*, 2007).

In this research we study the technology of microwave-assisted extraction and three conventional extraction methods: steam distillation (SD), hydrodistillation (HD) and solid-liquid extraction (SLE). In the application of SLE was analyzed the time and solvents, in the case of SD and HD the time was evaluated, while an experimental orthogonal design of five factors (tempera-

ture, stirring, time, ethanol solution, liquid-solid relation) was used to investigate the effects of parameters of microwave-assisted extraction. The novel of this work was to optimize the eugenol production by MAE. In this sense, Taguchi method was chosen because reduce the number of experiments like that amount of chemicals and time and a standard orthogonal array L-25 was used to examine a five factors system at five levels. Based on experimental parameters the operating conditions that ensure the optimal recovery of eugenol were evaluated.

II. METHODS

A. Plant Material

The buds of clove (*Eugenia caryophyllata*) were obtained from the same batch of a local producer in the province of Córdoba, Argentina. Prior to extractions, buds of clove were dried at 40 ºC in stove until constant weight, ground in a mortar and stored in plastic bags at room temperature, protected from light. The moisture content of dried clove buds was 14.0±0.5%.

B. Extraction Methods

Hydrodistillation (HD)

Clove (30 g) was submitted to HD with a Clevenger-type apparatus. The studied parameter was extraction time. The essential oil/water mixture was collected in an Erlenmeyer and then was subjected to extraction with dichloromethane in order to pass the eugenol to the organic phase. Sodium hydroxide 5% was added to the organic phase so the salt of eugenol was solubilized in water. The last phase was washed with dichloromethane to eliminate the organic component that could be remaining. Hydrochloric acid 10% was added to the aqueous phase to reconstitute a phenol, and dichloromethane was added to extract it. Later, sodium sulfate was added to the organic phase, the mixture was filter. Solvent was removed by roto-evaporator. As a result, pure eugenol was obtained.

Steam Distillation (SD)

A simple laboratory apparatus that consists of a steam generator, a 500 mL round-bottom flask, a collected vessel, a refrigerant and a tube Florentine, was used to perform the SD. Water was heated to produce steam which flowed through the round-bottom flask which contained the sample (30 g). The mixture of essential oil and water was condensed as it passed through the refrigerant and separated in the tube Florentine. The studied parameter was extraction time. Eugenol was obtained to the same methodology described in HD.

Continuous Solid-Liquid Extraction (SLE)

In this case, two equipment were used, Soxhlet and Twisselman extractors. To select the optimal solvent to extract the compounds of interest, initially, the experiments were carried out with solvents of different polarities during the same period of time. Once the optimal solvent was identified, the effect of extraction time was analyzed.

The sample (30 g) was transferred into a filter paper and inserted into to the corresponding equipment. The plant material was extracted to different time with 150 mL of selected solvent (ethanol). Vapors generated were condensed upon contact with the refrigerant, dripping through the sample into the boiling solvent. Once the extraction has been reached, solvent was recovered. Eugenol was obtained to the same methodology described in HD.

Microwave-Assisted Extraction (MAE)

Sample extractions were carried out in an Anton Paar Monowave 300. For each experimental run, 1 g of cloves powder was used. Extractions were performed under different extraction conditions, modifying the range of temperature variation (30 to 70°C), the range of stirring (240 to 1200 rpm), the range of time (5 to 25 min), the range of solution of ethanol (concentrations greater than 20%), and the range of liquid-solid ratio (7 to 19 mL/mg). The established values were chosen under the following criteria: ethanol is a good absorber of microwave radiation and clove oil is soluble in this, the liquid-solid ratio is important because an optimum ratio ensures homogeneous and effective heating, the chosen range was based on the capacity of the vial. Excessive solvent causes poor microwave heating as the microwave radiation would be absorbed by the solvent and additional power is required. Low ratio of solvent in solid promotes mass transfer barrier as the distribution of active compounds is concentrated in certain regions which limits the movement of the compounds out of cell matrix (Zhang *et al.*, 2011). After completion of the extraction, vials were allowed to cool at room temperature. The products were closed tightly and were stored at 8°C prior to further analyses. Eugenol was obtained to the same methodology described in HD.

C. Characterization

The concentrates were analyzed by a Perkin Elmer gas chromatograph (GC) equipped with an on-column injector, a flame ionization detector (FID), an PE-5% capillary column (DF: 0.53 µm, ID: 0.55 µm, L: 30 m), using nitrogen as carrier gas, to determinate the percent total peak-area of aroma compounds. Oven temperature was programmed to 80 °C for 2 min, then programmed heating from 80 °C to 230 °C at a rate of 6 °C/min, and at 230 °C for 2 minutes. Injector and detector temperatures were 230 °C. The carrier gas was adjusted to a linear velocity of 24 mL/min. Methyl salicylate was used as internal standard (Wenqiang *et al.*, 2007).

GC-MS analysis were performed on a gas chromatograph Shimadzu GC-QP5000 mass spectrometer equipped with a standard non-polar capillary column (DF: 0.25 µm, ID: 0.25 mm, L: 30 m) in chromatographic conditions similar to GC. The carrier gas, helium, was adjusted to a linear velocity of 1 mL/min. The identification of compounds from the extracts was made based on fragmentation patterns together with matching of the mass spectra which obtain from each sample and compared with those in the NIST Mass Spectral Library (Katata-Seru *et al.*, 2017).

D. Yield calculations

From the results obtained of extractions and subsequent

Table 1. Orthogonal design L_{25}

Run	Tempe-ature (ºC)	Stirring (rpm)	Time (min)	Ethanol Sol (%)	L:S Ratio (mL/mg)
1		240	5	20	7
2		480	10	40	10
3	30	720	15	60	13
4		960	20	80	16
5		1200	25	100	19
6		240	10	60	16
7		480	15	80	19
8	40	720	20	100	7
9		960	25	20	10
10		1200	5	40	13
11		240	15	100	10
12		480	20	20	13
13	50	720	25	40	16
14		960	5	60	19
15		1200	10	80	7
16		240	20	40	19
17		480	25	60	7
18	60	720	5	80	10
19		960	10	100	13
20		1200	15	20	16
21		240	25	80	13
22		480	5	100	16
23	70	720	10	20	19
24		960	15	40	7
25		1200	20	60	10

[Sol = solution; L:S = Liquid:Solid]

analysis of GC, the yield of essential oil (Y_{EO}) was calculated by Eq. 1, yield of eugenol (Y_E) was calculated by Eq. 2, and yield of global process (Y_P) by Eq. 3 for each of the method applied. The values were expressed as mean±standard deviation (n=2).

$$Y_{EO}\ (\%) = \frac{mass\ of\ EO\ (g)}{mass\ of\ cloves\ (g)} . 100\ \% \quad (1)$$

$$Y_E\ (\%) = \frac{mass\ of\ eugenol\ (g)}{mass\ of\ EO\ (g)} . 100\ \% \quad (2)$$

$$Y_P(\%) = \frac{mass\ of\ eugenol\ (g)}{mass\ of\ cloves\ (g)} . 100\% \quad (3)$$

E. Experimental design and statistical analysis

Experiments of MAE were performed according to an experimental design of Taguchi of five levels and five factors (L25) as shown in Table 1. This methodology provides a complete knowledge of each factor and its effect on yields. Taguchi technique analyzes the results by utilizing a statistical indicator referred as the signal-to-noise ratio, (S/N ratio) that translates experimental information into single value which shows presence of disparity (Kohli *et al.*, 2018). The analysis of variance (ANOVA) was used to investigate which factors had a remarkable result on the response parameters and given optimum working conditions.

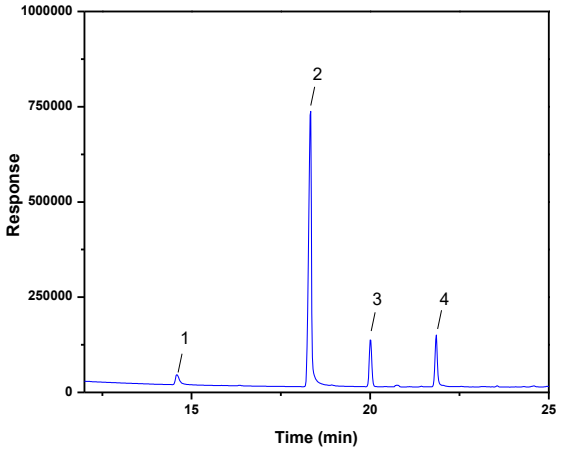

Figure 1: GC chromatogram obtained from analysis of oil clove. [(1) methyl salicylate (the internal standard), (2) eugenol, (3) β-caryophyllene and (4) eugenol acetate].

III. RESULTS

A. Chemical analysis of samples

Considering theoretical data on the content of essential oils in vegetable species, up to 18 w% of essential oil could be obtained from clove flowers, being its main components eugenol (89%), eugenol acetate and β-caryophyllene (5-15%) (Cortés-Rojas, Fernandez de Souza and Oliveira, 2014). Although the composition of the main components matches with data reported in the bibliography, such high experimental values in the recovery of essential oil have not been reported.

From GC/GS-MS analysis of clove essential oil samples obtained by means of extraction processes, three compounds have been identified: eugenol, β-caryophyllene and eugenol acetate. Taking into account a relative abundance of these compounds, it was found that eugenol is the main component. A typical chromatogram of essential oils analysis is shown in Figure 1. Under optimal MAE conditions, 80-92% of clove essential oil is eugenol, with 4-10% β-caryophyllene and 8-14% eugenol acetate; even though lower concentrations of these compounds have been determined in all of the samples, maintaining the proportion between them. The results found in our studies are within the range of concentrations and proportions of the components reported by other authors in the analysis of clove oils (Jirovetz *et al.*, 2006; Guan *et al.*, 2007).

B. Conventional Extraction Methods

Conventional methods present limitations when analyzing the effect of variables that can affect the process of

Table 2. Results obtained by conventional extraction methods

T (h)	Hydrodistillation			Steam distillation			Continuous solid-liquid extraction			
	Y_{EO} (%)	Y_E (%)	Y_P (%)	Y_{EO} (%)	Y_E (%)	Y_P (%)	E	Y_{EO} (%)	Y_E (%)	Y_P (%)
2	9.02±0.79	1.24±0.31	0.11±0.04	6.80±0.08	0.65±0.18	0.04±0.01	T	36.57±0.41	10.92±0.07	3.99±0.07
							S	32.67±1.10	9.69±0.19	3.16±0.12
4	11.52±0.54	1.53±0.21	0.22±0.03	8.84±0.12	5.75±0.17	0.51±0.02	T	41.60±0.76	4.65±0.19	1.93±0.11
							S	29.66±0.98	2.51±0.32	0.75±0.12
6	14.18±0.51	2.39±0.39	0.34±0.07	49.51±0.51	0.63±0.08	0.31±0.04	T	32.89±0.44	3.42±0.32	1.13±0.12
							S	27.48±1.22	3.38±0.39	0.93±0.15

[T = time; E = equipment; T = Twisselman extractor; S = Soxhlet extractor]

Table 3. Experiment layout by L_{25} orthogonal array and response values for yields (Y) results

Run	Yields of Essential Oil (Y_{EO})				Yields of Eugenol (Y_E)				Yields of Global Process (Y_P)			
	$Y1_{EO}$ (%)	$Y2_{EO}$ (%)	MSD	S/N	$Y1_E$ (%)	$Y2_E$ (%)	MSD	S/N	$Y1_P$ (%)	$Y2_P$ (%)	MSD	S/N
1	41.96	41.86	1756.45	32.45	2.93	2.83	8.30	9.19	1.23	1.18	1.46	1.64
2	45.84	45.81	2099.93	33.22	8.17	8.01	65.46	18.16	3.75	3.67	13.75	11.38
3	39.07	39.17	1530.38	31.85	26.95	26.82	722.77	28.59	10.53	10.51	110.62	20.44
4	29.27	29.31	857.90	29.33	4.57	4.15	19.05	12.80	1.34	1.22	1.63	2.13
5	31	31.05	962.55	29.83	91.11	91.02	8292.96	**39.19**	28.24	28.26	798.15	**29.02**
6	25.26	25.32	639.59	28.06	5.36	5.45	29.23	14.66	1.35	1.38	1.87	2.72
7	19.02	18.95	360.43	25.57	25.38	25.44	645.60	28.10	4.83	4.82	23.27	13.67
8	59.65	59.75	3564.09	**35.52**	5.09	5.16	26.28	14.20	3.04	3.08	9.37	9.72
9	30.03	30.12	904.51	29.56	4.51	3.18	15.23	11.83	1.35	0.96	1.38	1.39
10	47.14	46.98	2214.65	33.45	2.91	3.07	8.95	9.52	1.37	1.44	1.98	2.97
11	55.9	56.13	3137.69	**34.97**	57.81	57.88	3346.31	35.25	32.32	32.49	1049.98	**30.21**
12	49.67	49.82	2474.57	**33.93**	2.94	2.10	6.52	8.15	1.46	1.05	1.61	2.08
13	55.66	55.89	3110.86	**34.93**	2.40	2.15	5.18	7.14	1.33	1.20	1.61	2.07
14	52.38	52.97	2774.74	**34.43**	6.63	6.69	44.38	16.47	3.47	3.54	12.31	10.90
15	56.12	56.02	3143.85	**34.97**	2.17	2.38	5.19	7.15	1.22	1.33	1.63	2.13
16	33.85	33.95	1149.21	30.60	40.84	40.78	1665.57	32.22	13.83	13.84	191.41	22.82
17	44.55	44.18	1968.29	32.94	69.79	69.87	4876.50	**36.88**	31.10	30.87	959.89	**29.82**
18	42.85	42.76	1832.27	32.63	33.86	33.94	1149.15	30.60	14.51	14.51	210.56	23.23
19	14.32	14.64	209.70	23.22	90.48	90.53	8191.60	**39.13**	12.96	13.25	171.83	22.35
20	47.96	48.04	2304.00	33.62	2.70	2.75	7.44	8.71	1.30	1.32	1.71	2.34
21	19.45	19.79	384.97	25.85	30.82	30.75	947.58	29.77	5.99	6.09	36.47	15.62
22	22.02	22.42	493.77	26.94	79.60	79.72	6345.44	**38.02**	17.53	17.87	313.30	24.96
23	58.13	57.99	3370.97	**35.28**	63.63	63.56	4044.50	**36.07**	36.99	36.86	1363.41	**31.35**
24	56.47	56.67	3200.17	**35.05**	11.39	11.48	130.72	21.16	6.43	6.51	41.84	16.22
25	30.36	30.23	917.79	29.63	30.96	31.17	965.08	29.85	9.40	9.42	88.57	19.47

extracting essential oil from a matrix. In the three unconventional methods evaluated in this work, i.e. hydrodistillation, steam distillation and solid-liquid extraction, the variables of temperature, matrix mass and solvent volume are both conditioned by the properties of the stripping-extraction solvents used, and by the dimensions of the equipment in which they are used.

Under these conditions, the only variable to be evaluated is the extraction time, which was analyzed at three levels: 2, 4 and 6 h. Trials were carried out at extraction times greater than 6 h, whose results did not show higher yields than those obtained at 6 h of the process. This could indicate that the extraction times greater than 6 h would not increase the recovery of the essential oil and therefore the operational yields.

Table 2 shows the essential oil, eugenol and process yields obtained by these methods, at the three levels of the times selected. It should be noted that both in HD and SD, it is water that is used as a solvent and therefore, the process temperature is limited to its boiling point.

As can be seen for HD, the maximum EO, E and process yields were obtained at 6 h of extraction. It can be observed that the EO recovery yield increased 28% in 4 h and 57% in 6 h in relation to the yields obtained at 2 h. In turn, it is observed that the EO extraction process was accompanied by the enrichment of its content in eugenol, increasing the yield to eugenol in 6 h within values of the order of 93% in relation to the values obtained after 2 h of extraction. As a result of the process, the Y_P showed an increase of higher than 200% at 6 h of extraction, in relation to the values found at 2 h.

In the case of SD, the maximum EO yields were obtained at 6 h, while the maximum eugenol and process yields were obtained at 4 h. With the increase in the extraction time, the EO yield raised. However, the process was not accompanied by an enrichment of eugenol. This could indicate that, under the extraction conditions throughout the process, the eugenol could have been degraded by oxidation and/or polymerization of eugenol (Kapadiya *et al.*, 2018).

In the case of SLE, initially, the experiments were carried out with solvents of different polarity at a same time (ethanol, water, n-hexane), in order to compare the yields. The higher yield of eugenol was obtained with ethanol. Once the solvent was selected, the effect of the time in extraction process was evaluated with two different equipment: Twisselman Extractor (T) an Soxhlet Extractor (S). As it is showed in Table 2, the results indicate that regardless the type of extractor used, the results were similar. Between 2 to 6 h of extraction, the EO yields were between 27-42%. Within just 2 h of extraction, the best Y_E and Y_P were obtained. Longer extraction times show a reduction in yields of eugenol and process, which would indicate degradation of eugenol by oxidation and polymerization during extraction times longer than 2 h (Kapadiya *et al.*, 2018).

From the results presented in Table 2, it is possible to conclude that under the conventional extraction methods studied, Y_P of 3.0-4.0% are obtained by continuous S-L extraction at a process time of 2 h. The essential oils obtained by this method presented higher eugenol contents, even those recovered by the HD and SD procedures at different time tests.

C. Microwave-Assisted Extraction and statistical analysis

In the Microwave Assisted Extraction (MAE) technique there are several variables that influence the process of extracting essential oil from cloves. For our case under study, the factors evaluated were: temperature, stirring speed, extraction time, extraction solvent and matrix mass/solvent volume ratio (liquid: solid ratio). According to Taguchi, the design of the experiments was carried out including these 5 factors at 5 levels. The selected levels for each factor were considered on the basis of previous experiences of the working group.

On the basis of the results obtained from the Taguchi experiment design, the mean square standard deviation (MSD) and the signal-to-noise ratio (S/N) were calculated. The S/N ratio is a performance measure developed by Taguchi to choose the best levels as to deal with noise. In other words, the S/R ratio is a robustness measure used to identify control factors that reduce the variability of the extraction process by minimizing the effects of factors that cannot be controlled (noise factors). Table 3 shows the results of the yields, MSD and S/N ratio, for each experimental design conditions.

By analyzing the results of the S/N ratio for the Y_{EO}, it is observed that the maximum values are between 33.93-35.52, which correspond to tests 8, 11, 12, 13, 14, 15, 23 and 24 (marked in bold), which correspond to the best EO performance obtained with values between 49.7-56.7. Most of the best results are observed between tests 11-15, which are carried out at 50°C. A better analysis of the results is obtained by analyzing the total values of the S/N ratio for each level of the evaluated factors, which is presented in Table 4. The highest values of the S/N ratio indicate that optimal conditions for the best Y_{EO} performance is: 50°C, 1200 rpm, 15 min, 40% ethanol solution and 7 mL/mg of S: L ratio.

At the same time, the experimental yields of eugenol were analyzed and the results are presented in Table 3. From the S/N ratio, it is observed that the maximum values are in the range of 36.07-39.19, with Y_E in the range of 63.56-91.11, whose conditions would correspond to that of tests 5, 17, 19, 22 and 23. Only test 23 equates with the best results obtained with Y_{EO}. For a better analysis of the results, we examined the total values of the S/N relations for each level of the different factors in Table 4. The results indicate that the optimal conditions to obtain maximum yields at E are: 70 ° C, 480 rpm, 25 min, 100% ethanol solution and 19 mL/ mg liquid-solid ratio.

The results that corresponds to the process yields are shown in Table 3 and Table 4. Based on the analysis of the S/N relationships, the best process yields correspond to tests 5, 11, 17 and 23. In these cases the ranges of S/N and Y_P are between 29.02-31.25 and 28.24-36.99%, respectively. Only the conditions of tests 17 and 23 matches the best performances previously analyzed. Based on the analysis of the total values of the S/N ratios for each level of the different factors, the optimal conditions to obtain the best performance of Y_P are: 70°C, 720 rpm, 15 min, 100% ethanol solution and 19 mL/mg liquid-solid ratio. Similar levels of the factors proved to be optimal when analyzing Y_{EO} and Y_E.

Table 4. Total S/N ratios corresponding to results of yields

Factor	Levels	S/N ratio		
		Y_{EO}	Y_E	Y_P
Temperature (ºC)	30	156.69	107.93	64.61
	40	152.16	78.30	30.46
	50	**173.24**	74.16	47.39
	60	153.02	147.55	100.56
	70	152.75	**154.87**	**107.61**
Stirring (rpm)	240	151.93	121.08	73.01
	480	152.60	**129.31**	81.91
	720	158.14	116.60	**86.81**
	960	151.60	101.39	52.99
	1200	**161.51**	94.42	55.93
Time (min)	5	159.90	103.81	63.70
	10	154.75	115.17	69.93
	15	**161.06**	121.81	**82.87**
	20	159.02	97.20	56.22
	25	153.12	**124.80**	77.92
Ethanol Sol (%)	20	164.85	73.94	38.78
	40	**167.26**	88.20	55.46
	60	156.91	126.45	83.36
	80	148.26	108.42	56.78
	100	150.47	**165.79**	**116.26**
Liquid:Solid Ratio (ml/mg)	7	**170.93**	88.59	59.52
	10	160.01	125.68	85.69
	13	148.31	115.15	63.45
	16	152.88	81.34	34.22
	19	155.72	**152.04**	**107.76**

Table 5. Statistical F of experimental data obtained for Taguchi design.

Factor	F-value		
	Y_{EO}	Y_E	Y_P
Temperature	1.60	2.65	0.67
Stirring	1.17	0.25	0.24
Time	0.20	0.69	0.23
Ethanol Sol	1.00	4.06	0.74
L:S Ratio	1.26	1.09	0.61

In order to analyze which of the factors under studies have a greater influence on the extraction process, we evaluate the values of the F statistic. This test allows us to assess the effect of the experimental factors on the average response, compared to the experimental error. A high value of F associated with a factor indicates that such a factor has a greater effect on performance, in relation to the other factors in question; even if a low value of the F associated with a factor, indicates a greater weight of such a factor on the results.

Table 5 shows the values of statistic F obtained with different factors in relation to the experimental results of EO, E and process yields. For Y_{EO} and Y_P, the lowest F value corresponds to that of the factor of time and indicates that this variable is the least influential on the process performance. In the case of the eugenol yield, the lower value of F corresponds to stirring. For the whole of the returns of EO and E, the temperature factor is predicted to be the most influential.

From the results analyzed it is possible to conclude that high EO, E and process yields would be obtained

Table 6. Best yields of eugenol obtained by different methods

Method	Conditions	Y_E (%)
HD	6 h – water – 100 ºC	2.39±0.39
SD	5 h – water – 100 ºC	5.75±0.17
SLE	6 h – ethanol – 78 ºC	10.92±0.07
MAE	0.4 h – ethanol – 70 ºC	92.43±0.83

under conditions established by experience 23, although the factors can be properly selected on the basis of the established process requirements.

It is of interest to obtain essential oil of clove with high concentrations of eugenol, it is for this reason that we consider the optimal conditions established by the Taguchi design to obtain maximum Y_E. Tests were carried out under these conditions, finding Y_E of 92.43±0.85.

C. Comparisons between yields obtain by different methods

The selection of an extraction method and its parameters will depend on the objective of production, costs, safety and environmental impact. As we said previously, it is a requirement for our work to obtain clove essential oil with high concentrations of eugenol. Table 6 shows the best Y_E results found by the extraction methods evaluated in this work, including the experimental conditions in which they were obtained. From these results it can be observed that the lowest Y_E were obtained by the HD and SD processes, and the MAE process substantially exceeded these yields. MAE is a method that offers huge advantages over conventional methods, mainly the reduction in the times of extraction and the volume of solvent extraction. Although the best eugenol yields were a consequence of high Y_{EO} for the MAE experiments, this was not reflected on the tests carried out through unconventional methods. The extracts obtained by using non-conventional methods were blackish brown due to the presence of oxidation and polymerization products as a consequence of thermal degradation; characteristics that were not presents in the essential oils obtained by MAE.

IV. CONCLUSIONS

This investigation is focused in the study of different parameters of extraction techniques to obtain cloves oil and eugenol through purification of EO. The experimental design of Taguchi allows optimizing the parameters of the MAE process. The optimized conditions for obtaining of eugenol based on the five factors were 70 °C of temperature, with 480 rpm of stirring, 25 min of process, 100 % of solvent and a liquid-solid ratio of 19 mL/mg. That means, MAE offers a production of eugenol with lower energy cost and solvent, making it an environmentally friendly technique.

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