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Design of experiment (D.O.E) by Taguchi model for optimization of different process parameters for LSPR system to detect VCO adulteration

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Abstract
Virgin coconut oil (VCO) being one of the costly oils is prone to be adulterated with cheaper oils *viz*., coconut oil (CO). Attempt were made to develop a new innovative way to detect the adulteration in VCO using Localized surface plasmon resonance (LSPR) system. Optimization of certain process parameters is an prioritize criteria for detection by LSPR system for further development of the calibration curve. The traditional method for optimization involved the study of one variable at a time, which in turn required a large number of experimental runs. The Design of experiments (D.O.E) by Taguchi design employed here for optimization of LSPR system to achieve maximum reliability with a minimum experimental run. The process parameters *viz*., flowrate, surfactant and pH were selected for the optimization of the LSPR system to detect the VCO adulteration with CO in PBS buffer. For obtaining a maximum response a large signal to noise ratio was applied. An optimal condition for LSPR system was obtained at a Flowrate (200µl/min), pH (7.0) and surfactant (Tween 20) with a significant difference (p<0.05). A linear regression model with high significancy was developed and the model was validated. The results suggested that the Taguchi method can be used as an effective optimization method for LSPR system for VCO adulteration detection by further development of the calibration curves.

Keywords: Taguchi method, optimization, adulteration, virgin coconut oil, localized surface plasmon resonance

1. Introduction

Virgin coconut oil (VCO) being one of the costliest oils compared to other edible oils has emerged as a major issue for adulteration with other cheaper oils. VCO has being reviewed to adulterate with other edible oils which includes coconut oil (CO), paraffin oil, palm oil and mustard oil (Amit, Jamwal, Kumari, Kelly, *et al.*, 2020) [1]. Adulteration of edible oils causes genuine medical issues like Spanish harmful oil disorder or Spanish olive oil disorder by offering non-palatable rapeseed oil as an eatable rapeseed oil and as olive oil (Clemente and Cahoon, 2009).

There are various instrumental methods for detection of the adulteration of VCO with other edible oils *viz*., Fourier transform infrared (FTIR) spectroscopy, Differential scanning calorimetry (DSC) (Mansor, Tengku Salwani Tengku *et al.*, 2011) [16] etc. However, all the above listed instrumental techniques needs more man power, time as well as a in depth chemical knowledge (Meenu *et al*., 2019) [18]. Therefore, a new technique for detection of adulteration in VCO should be encouraged which can be helpful for food processing sectors.

In the recent era, through the tremendous growth in optical science, Localized surface plasmon resonance (LSPR) has come into play due to their simplicity in operation and cost (Haes & Duyne, 2004) [9]. In LSPR system, the oscillation of free electrons between the edible oil and the gold sensor chip cause shifts in the response peak. The change in the shifts can be measured to verify the amount of adulteration in VCO with CO and MO.

In LSPR system, or any other instrumental analysis optimization is an important aspect to cover, before running samples and developing the calibration curve to maintain the system accuracy (Kabir *et al*., 2020) [10]. The important process parameters for edible oil adulteration detection includes maintaining pH of buffer, surfactant in which the oil dissolves and the flowrate of injection of the oils. A study done by Bremer *et al*., (2009) [3] may give us a rough idea about the LSPR system being used to detect the adulteration in hazelnut oil using Phosphate Buffer Saline (PBS) which is widely used with LSPR system. Traditional method to optimize a process included first identification of the various independent variables and then performing experiments “one variable at a time” (OVAT) system.

These involved carrying of many experiments and the interpretation between the variables could not be found (Rao *et al*., 2008) [25]. The optimization of the different experimental process parameters nowadays is mostly done using different design of experiments (D.O.E) methods (Weissman *et al*., 2015) [28]. Design of experiment (D.O.E) is a multipurpose mathematical tool used for planning and performing experiments, as well as to identify, analyze and interpret important data and to find how the input variables are related to that of the output variables (Durakovic, 2017) [7]. Ronald A. Fisher was the first person who conducted D.O.E rather than traditional methods to avoid getting too long results and analyze the data in presence of external factors like temperature, soil, rainfall) in his experimental work which aimed to increase the yield of crops in U.K and concluded D.O.E. (Durakovic, 2017) [7]. Therefore, D.O.E is the best for optimization of different process parameters using less experimental runs and avoid using too long data.

Various commercial statistical free software *viz*., Minitab, SPSS, Origin Pro, Prisma etc., are being used widely for design and analyze D.O.E. Moreover, Microsoft Excel, a well-known tool can also be used for D.O.E using standard procedures and formulas (D. Granato *et al*., 2014) [6]. Two important statistical methods *viz*., linear regression and analysis of variance (ANOVA) are important to understand before performing D.O.E for any process. Moreover, the basic practical steps for performing D.O.E includes: (a) listing down the problems/objectives (b) define the objectives (c)selection of independent variables along with determining levels and factors (d) determination of the experimental design type (e) performing experiment with the designed matrix (f) data analysis (e) Conclusion.

There are many approaches for Design of experiments for optimization *viz*., Factorial designs with one, two, three and more factors, full factorial design and fractional factorial design. These all approaches give quite complexity in the experiments as it considers all the number of possible combinations and increases the experimental runs which requires a lot of raw materials, expensive and time consuming (Rao *et al*., 2008; Durakovic, 2017) [25, 2]. Considering all these difficulties, Dr. Genici Taguchi concluded a special factorial design i.e Taguchi model, which significantly could reduce the experimental runs and could cover no of applications.

Taguchi technique is a powerful designing method which exposes the process to various levels of parameter design and procedures to get best levels of product/process quality with minute variations (Ealey Lance A, 1994) [8]. The Taguchi approach is based upon the signal to noise (S/N) ratio (log function for response for selected parameters) and analysis of variance (ANOVA) to form the best design in an experiment (Karna *et al*., 2012) [11]. The S/N ratio integrates with the model and predicts the outcome to attain enhance the performance in the process. In recent scenario, Taguchi model have been used for optimization of different drying conditions for extracted ginger oil (Chen *et al*., 2011) [4], optimization for fabricated lactalbumin nanoparticles (Mehravar *et al*., 2011) [23], optimization of ultrasound assisted extraction for bioactive (Salacheep *et al*., 2020) [26], optimization of gluten free spaghetti (Mayasti *et al*. 2019) [17]. The advantages of using Taguchi model includes low cost and time, determines each factors, predicts optimal conditions, defines error with a minimum experimental runs (Koorand *et al*., 2018) [13].

There is no literature reported on optimization of LSPR system for adulteration detection using Taguchi method in VCO adulterated with CO. Therefore, this study was designed to examine the effect of selected parameters important for getting good response in LSPR system with minimum experimental runs.

2. Material and Methods

2.1 Raw materials and apparatus

VCO and CO were purchased from M/s Pavithra Kera Pvt Ltd., Palakkad, Kerala. All reagents, buffers and surfactants used were purchased from M/s Sigma Aldrich-Merk. All reagents used for analysis were of analytical grade. LSPR system were purchased from M/s Nicoya Lifesciences Inc., Kitchener, Canada.

2.2 Design of experiment (D.O.E) by Taguchi model for optimization of pH, surfactant, flowrate in LSPR system

Different process parameters important for adulteration detection in VCO *viz*., pH of the PBS Buffer, surfactant to dissolve the adulterated VCO in PBS Buffer, injection flowrate of the adulterated samples of VCO with CO was selected for optimization of LSPR system as per review. Experimental runs were designed using adulterated VCO sample with CO in PBS Buffer through the Taguchi orthogonal array method of L16 (4^4) using MiniTab 17.0 software with different parameters and levels of Ph, surfactant and flow rate shown in Table 1. Then, analysis of variance (ANOVA) was employed for all the runs to determine the significance of parameters for the response curve from LSPR system. S/N ratio was calculated at each parameter level to find the optimal level of each parameter to eliminate the inappropriate response caused by the noise factors in the optimization process. In this experiment as maximum response is needed, larger S/N was applied, which was determined using the following equation (Ghiasi *et al*., 2021) [9].

$$S/N = -10 \log [1/n \Sigma (1/yi^2)] \quad \ldots (1)$$

where 'y' and 'n' are the observed data and number of observations, respectively.

Table 1: Process Parameters and Their Levels

Parameters	Levels			
pH	7	7.2	7.4	7.6
Surfactant	Triton-X	Brij-30	Tween 20	Tween 80
Flowrate	50	100	150	200

Table 2: Taguchi orthogonal array L16 (4^4) design of experiments

Runs	Variable		
	pH	Surfactant	Flowrate
1	7	Tx	150
2	7	B30	100
3	7	T20	200
4	7	T80	50
5	7.2	Tx	100
6	7.2	B30	50
7	7.2	T20	200
8	7.2	T80	150
9	7.4	Tx	150
10	7.4	B30	200
11	7.4	T20	50
12	7.4	T80	100
13	7.6	Tx	200
14	7.6	B30	150
15	7.6	T20	100
16	7.6	T80	50

2.3 Linear regression model analysis for Taguchi model
The linear regression equation was developed for the maximum current response observed. The current response was applied by following the S/N ratio. Multiple correlation coefficient (R^2) was used to represent model descriptive quality.

2.4 Validation and residuals analysis for Taguchi model
The model was validated with experimental values for the given parameters. The relative error of current response and S/N ratio calculated by the observed value. The residual analysis was employed to find out if the model fits the statistical assumptions, i.e., that the residuals are random and normally distributed with a mean of zero and constant standard deviations, and that was done with a 95% level of confidence (Montgomery *et al*., 2012) [19].

$$\text{Relative error} = x = \frac{\text{Experimental value} - \text{Predicted value}}{\text{Predicted value}} \quad \ldots (2)$$

3. Results and Discussions
3.1 Design of experiments (DOE) for optimization of pH, surfactant, flowrate
The optimum levels of parameters such as pH (A), surfactant (B), flowrate (C) was investigated through the DOE method in adulterated VCO samples with CO in PBS buffer. A mixed level design of one parameter with two and three others parameters with three levels based on the L16 (4^4) in Minitab software, were conducted according to Table 2 with adulterated samples of VCO with CO in PBS Buffer. The main effects and interaction plots between the parameters are depicted in Figure 2.

As seen, at PBS buffer, pH 7.0 maximum response was obtained. As the pH of the PBS buffer increased from 7.0 to 7.6 the response was declined. This may be due to the fact that as the buffer becomes alkaline, it causes interaction with the materials on the sensor chip causing lower response in the LSPR system.

Oil being an immiscible liquid surfactant was used to dissolve the adulterated VCO with CO in PBS buffer. In case of the surfactant, Tween 20 (250µl) showed a maximum response compared to other surfactants Brij-30, Triton-X and Tween 80 in edible oil. This is due to the fact that Tween 20 as a surfactant recognizes a higher density phase which makes it more stable (Sheen *et al*., 2011) [27]. Similar results were reported by (Lin *et al*., 2010) [14] Tween 20 along with gold nano particle showed a stable response for detection of mercury in the drinking water samples. Stability of the surfactants depends on the stability factors or the orientation of the surfactants at the interface (Katakam *et al*., 1995) [12]. Moreover, no insoluble pellets or aggregation of Tween 20 was found in the sample solution.

Again, in case of injection flow rate for adulterated VCO samples with CO in PBS buffer, 200 microliter/minutes showed the highest response. The effect of different flow rate was studied with adulterated samples of VCO with CO Figure 2. by injecting in the LSPR system. Results revealed that in the flow rate of 150 and 100 microliter/minutes a negative drip was observed. The negative response may be due to the clogging of the oil samples in the flow injection channel.

In the interaction plots, if the lines are nonparallel, then there is an interaction between parameters and if the lines cross, strong interaction occurs (Kirati *et al*., 2019) [21].Figure 2 illustrates the strong interaction between parameters flow rate and surfactant and surfactant and pH.

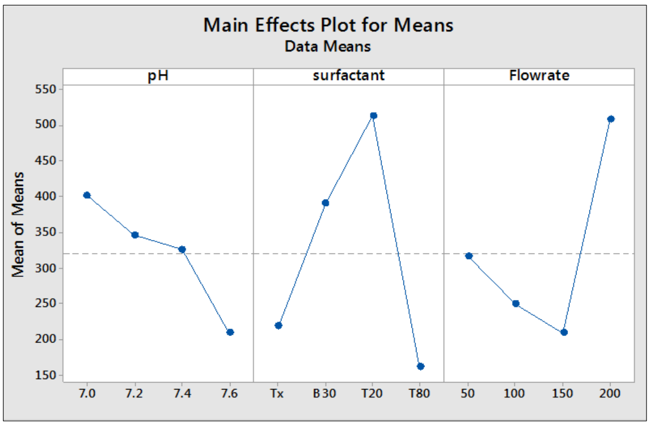

Fig 1: Main effects plot

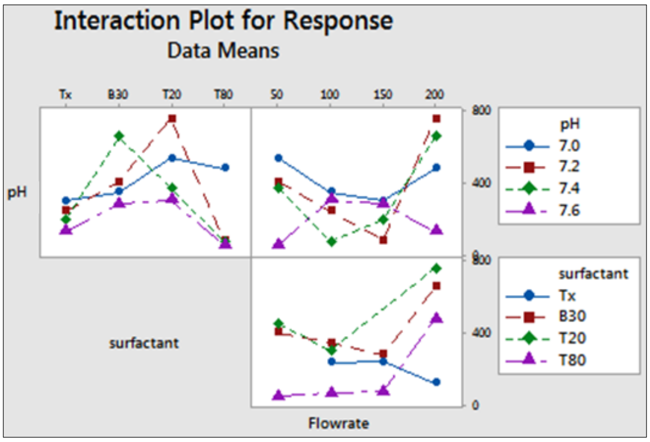

Fig 2: Interaction plot

Table 3 provides the response table, which compares the relative degree of impacts by ranking them according to delta values. The difference between the highest and lowest mean for each parameter was used to determine delta (Moozarm Nia *et al*., 2019) [22].The surfactant and flowrate appear to be the most significant that determine the peak response. The response is the least affected by pH.

Table 3: Response Table for Signal to Noise Ratios Larger is better

Level	pH	Surfactant	Flowrate
1	51.81	46.43	44.49
2	45.11	48.24	46.37
3	47.51	53.73	45.30
4	44.40	40.43	52.57
Delta	7.41	13.30	7.97
Rank	3	1	2

3.2 Analysis of variance
In Taguchi analysis, the ANOVA test is commonly used to characterize the effective parameters on the average response and S/N ratio. Table 4 shows the degree of freedom (DF), the sum of squares (SS), mean square (MS), F-value (F), and p-value based on S/N data. The ANOVA demonstrates the relative relevance of each parameter using the sequential (Seq) and adjusted (Adj) sum of squares, with the parameter with the highest SS having the main effect. SS values show that the surfactant parameter has the highest SS value, followed by flowrate and Ph.

Furthermore, the surfactant has the lowest p-value (0.011) and the pH had the largest p-value (0.156). The parameters such as surfactant and flowrate are found to be significant with

$p<0.05$ and whereas, the pH with $p>0.05$ was not significant. Therefore, the surfactant used for dissolving VCO with CO in PBS buffer has the greatest impact on the response curve, while the pH has the least impact. These features are considered key factors since even the smallest variations in any of them might result in substantial changes in performance.

Table 4: Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	3	78576	78576	26192	2.50	0.156
Surfactant	3	3109341	2924821	97494	9.31	0.011
Flowrate	3	191593	91593	63864	6.10	0.030
Residual Error	6	62862	62862	10477		
Total	15	643966				

3.3 Linear regression model
The model equation was developed for maximum current response. “Larger is the better” S/N was applied for current response using Equation 1. Equation 2 denotes the regression equation based on the results of S/N ratio.

Response = 320.625 +81.042 (pH 7.0) +25.458 (pH 7.2) + 4.875 (pH 7.4) -115.625 (TX) + 69.125 (B20) + 206.042 (T20) -84.958 (Flowrate 50) -71.208 (Flowrate100) -30.958 (Flowrate 150) …. (1)

(S/N ratio) = 47.2079 + 4.6044 (pH 7.0) - 2.1012 (pH 7.2) + 0.3046 (pH 7.4) – 2.2339 (TX) + 1.0319 (B30) + 7.9749 (T20) – 5.1660 (Flowrate 50) – 0.8345 (Flowrate 100) + 0.6406 (Flowrate 150) …. (2)

Multiple correlation coefficient (R^2) was used to represent model descriptive quality. The R^2 value of response and S/N ratio of models were 90.2% and 86.2% respectively. Because of the higher R^2 value, it can be ascertained that the experimental and predicted values are very close, and the regression model is highly significant.

3.4 Validation and residuals analysis
The model was validated with experimental values for the given parameters shown in Table 5. The relative error of current response and S/N ratio calculated for the given conditions was 1.65 and 0.22 respectively. The positive error may be due to the different affinity of the compounds present in the complex nature of the edible oils (Morales *et al*., 2002) [20]

Table 5: Validation of the model

	Parameters			Experimental	Predicted	Relative Error
	pH	Surfactant	Flowrate			
Response	7	T20	200	534.6667	201.083	1.65
S/N	7	T20	200	54.5573	44.4123	0.22

Figure 3 illustrates the residual analysis employed to find out if the model fits the statistical assumptions, i.e., that the residuals are random and normally distributed with a mean of zero and constant standard deviations, and that was done with a 95% level of confidence (Montgomery *et al*., 2012) [19]. The normal probability plot revealed that they are rather close to a straight line, meaning that the errors are normally distributed and the normality hypothesis is met (Figure 3 a). The variation of residuals as a function of fitted values is shown in Figure 3 b. The standardized residuals yielded a randomly distributed scattered point distribution within the range of ±4. The random scattering of residuals around the surface indicated that the model was appropriate, and the independence and constant variance assumptions were not infringed. Therefore, the Taguchi model developed for optimization of LSPR system can be further used for development of calibration curve using different percentage of adulterated VCO samples with coconut oil as well as other vegetable oils *viz*., mustard oil.

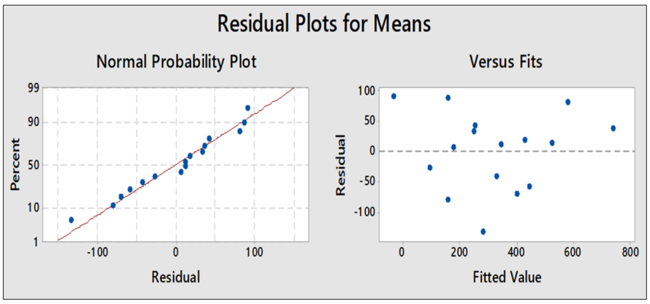

Fig 1: Residual plots

4. Conclusion
The interactions study between the different parameters important for LSPR system as per reviewed includes flowrate, pH and surfactant showed a good influence in the response of the LSPR system with adulterated VCO with CO in PBS buffer. Further, an optimal condition for adulteration detection of VCO with CO using LSPR system obtained was with using sample injection flowrate (200µl/min), pH (7.0) and surfactant (Tween 20). The most important and first ranking parameter was found to be the surfactant used to dissolve the adulterated oil in PBS buffer. The R^2 value of linear response model and S/N ratio of models were 90.2% and 86.2% respectively which shows high significancy. The relative error between the response of LSPR system(experimental) and the predicted response for the given optimal conditions was minimal 1.65 and 0.22 respectively which can be acceptable. This approach enables a great concept for effective optimization of the LSPR system for edible oil adulteration detection and further using of the system with varying concentration of adulterated samples of VCO with CO as well as with other oil *viz*., mustard oil.

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